

CERTAIN FACTORS AFFECTING VITAMIN B12 ABSORPTION
AS ASSESSED BY WHOLE BODY COUNTING

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SUMMARY

Many methods have been devised for measuring vitamin B12 absorption since the report of Heinle et al. (1952) using the faecal excretion method. However, though all of the currently accepted routine tests give good results in clinical practice, all are subject to a variety of inaccuracies, many of which cannot even be calculated. Furthermore, none is wholly suitable for research purposes, as all but the faecal excretion test are at best semiquantitative and the faecal excretion test itself is indirect, being subject to errors resulting from incomplete faecal collection.

Since the report of Reizenstein et al. (1961) on the use of whole body counting for measuring vitamin B12 absorption, this method has been increasingly used, at least for research purposes. The currently available machines are sensitive, quantitative measurements of absorption can be made directly, and the possible extent of the methodological sources of error can be measured. The purposes of the present work were to develop a whole body counting method for measuring vitamin B12 absorption, to assess the extent of the sources of error, to investigate the ranges of normal and abnormal absorption as well as the reproducibility of the results, to test the effects of food on these, and to relate vitamin B12 absorption to intrinsic factor secretory capacity where this was diminished.

Preliminary experiments were done to determine the best way of testing intrinsic factor secretory capacity. Investigations in

15 subjects showed that pentagastrin gave rise to fewer unpleasant side-effects than histamine. As 6.0 μ g/kg of body weight of pentagastrin gave an equivalent intrinsic factor output to that obtained with 0.04mg/kg of body weight of histamine, pentagastrin was used as the gastric stimulant of choice. Experiments were done to determine the best conditions for collecting gastric juice so as to ensure optimum survival of immunoassayable intrinsic factor; it was found that the most important point was to destroy pepsin, and that doing this by raising the pH of the gastric juice to 10 for 30 minutes did not damage intrinsic factor. Keeping gastric juice at room temperature or at 4°C also preserved intrinsic factor before pepsin had been destroyed.

In developing the whole body counting method a scanning bed geometry was used, as it was considered that this geometry would give the optimum results. Machine sensitivity, as defined below, was high, being 0.014 μ c or 1.4% of the test dose of radioactive vitamin B12 used. Furthermore, a comparison of two different machines, in which studies of the 100% value were done on 29 subjects and of vitamin B12 absorption values on 25 subjects, showed that inter-machine variability was minimal.

⁵⁷Co and ⁵⁸Co were used as radioactive labels. For ⁵⁸Co three energy ranges for counting were tested--0.40-0.65 Mev, 0.65-1.00 Mev, and 0.40-1.00 Mev--and the 0.40-1.00 Mev range was chosen as giving the best combination of sensitivity and variation. The coefficients of variation for the 100% values, tested in 29 subjects, was 3.8% for

58Co (16 subjects) and 6.9% for 57Co (13 subjects), which compares well with the results of other workers.

The excretion of radioactivity in the urine and stools during the week of the test was investigated. Of 11 subjects, 10 lost less than 3% of the test dose in the urine in one week, and 7 of these lost less than 1%. Daily faecal collections in 45 subjects showed that the mean faecal excretion of radioactivity was 0.1% of the test dose on the seventh day of the test, and that the day of maximal excretion of radioactivity fell before the fifth day in 93% of subjects. It was therefore decided that the final count could be done safely on the seventh day.

Profile scanning as a means of detecting unexcreted and unabsorbed radioactivity was tried in 24 subjects, 9 of whom had subnormal levels of absorption. A collimator setting of 5cm was found most suitable. In 21 subjects two peaks of radioactivity were seen during the week of the test; one which did not disappear was called a "liver peak," and one which disappeared coincidentally with excretion of radioactivity in the faeces was called an "intestinal peak." The "intestinal peak" could be reproduced by instilling 5-10% of the test dose into the rectosigmoid region. As one subject with normal absorption showed only a single peak throughout the week of the test, a one peak pattern could not be used to exclude the presence of unabsorbed radioactivity. However, the presence of a two peak pattern does indicate that all unabsorbed radioactivity has not been excreted.

Investigations using this method showed that when the vitamin B12 test dose was given in the fasting state, control subjects all absorbed 30% or more of the test dose. Five subjects with pernicious anaemia gave test results above this value; in 4 cases only one of two tests exceeded 30%, and in one case both tests exceeded 30%. When the test dose was given with food, all controls absorbed 47% or more of the test dose; one subject with pernicious anaemia exceeded this value on one of two test occasions. Subjects with achlorhydria who did not have pernicious anaemia very rarely gave test results below the normal range. Failure of faecal excretion of unabsorbed radioactivity was probably the most frequent cause of normal absorption values in pernicious anaemia subjects, but the possibilities of wrong diagnosis or occasional normal absorption of vitamin B12 in pernicious anaemia are discussed.

The reproducibility of test results in individual subjects was poor, especially in control and achlorhydric subjects. This was interpreted as indicating that the amount of vitamin B12 absorbed on different occasions in any subject can vary greatly. Reproducibility of results improved significantly in these two groups when the test dose was given with food; this may be the result of a combination of increased intrinsic factor secretion and slower gastric emptying when food was given. Variations in test results could also be striking in subjects with intestinal malabsorption or particularly partial gastrectomy; in these groups, results in both the normal and malabsorption ranges could be obtained in the same subject.

It was noted that mean vitamin B12 absorption was greater when the test dose was given with food in control, achlorhydric, and especially partial gastrectomy subjects. The significance of these results was hard to evaluate, as the same subjects were not always given tests both with and without food. The findings are, however, discussed in relation to similar results obtained by other workers.

A comparison was made between the results of vitamin B12 absorption tests done by the faecal excretion and whole body counting methods in 56 subjects. This showed that absorption results were higher using the faecal excretion method; that this was almost certainly due to failure of adequate collection of faeces was indicated by the disparate results obtained in some subjects with pernicious anaemia. It was considered that inadequate collections could have occurred in about 23% of subjects.

In subjects who produced less than 1000ng units of intrinsic factor in one hour after gastric stimulation, at least where absorption tests were done in the fasting state, χ^2 analysis showed that there was a significant relation between vitamin B12 absorption and intrinsic factor production. However, there were also a number of individual exceptions to this finding. Some achlorhydric subjects without pernicious anaemia absorbed vitamin B12 normally yet produced less than 100ng units of intrinsic factor. Other subjects with pernicious anaemia produced more than 500ng units of intrinsic factor and yet had very poor vitamin B12 absorption. It was concluded that an absolute differentiation between achlorhydria with and without pernicious anaemia cannot be made from the intrinsic factor secretory capacity alone.

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INTRODUCTION

The purpose of this introduction will be to review the normal absorption of vitamin B12 into the body, as well as the nature, source and daily need for this vitamin. Emphasis will be laid on the methods which have been developed to measure the absorption of this vitamin and in particular on work which has already been done on the development of methods using the principle of whole body counting. No attempt will be made to discuss the metabolism of vitamin B12 once it has entered the body.

VITAMIN B12

Although the term vitamin B12 may be used to refer specifically to the substance cyanocobalamin, there are many other analogues of this material which have vitamin B12 activity biologically; these substances may be referred to collectively as the cobalamin analogues. The vitamin B12 molecule consists of two major parts. One of these is the corrin ring, which resembles a porphyrin, and when this ring contains a cobalt atom and carries certain specific side-chain groups, it becomes known as cobamide, as in vitamin B12. The second major part of the molecule is a nucleotide set almost at right angles to the corrin ring. Finally, also attached to the corrin ring through the cobalt atom, there is a ligand. In this way there occurs a group of analogues,

varying with respect to the ligand, some of which are shown in Table I.

Although cyanocobalamin was the first analogue to be isolated (Rickes et al. 1948, Smith and Parker 1948), it is not the main dietary source of vitamin B12, and it is likely that it is usually formed as a result of the chemical decomposition of deoxyadenosylcobalamin (Toohey and Barker 1961). Deoxyadenosylcobalamin itself is the predominant form of vitamin B12 analogue in food, having been found in a variety of animal livers, including that of man (Toohey and Barker 1961); however, smaller amounts of other cobalamins may also be found, such as methylcobalamin, cyanocobalamin, and hydroxocobalamin (Lindstrand and Stahlberg 1963, Rosenblum et al. 1963, Lindstrand 1964). Hydroxocobalamin, itself biologically inactive, may be an intermediate, and cyanocobalamin can be converted to other forms, such as adenosylcobalamin, in the liver and kidney (Reizenstein 1967). The cobalamins found in food are bound to cellular protein (Cohn et al. 1928).

From the viewpoint of studies on vitamin B12 absorption, the most important advance was the discovery of ways to introduce radioactive cobalt into the structure of the vitamin. This was first achieved by Chalet et al. (1950), who labelled vitamin B12 with ^{60}Co to a specific activity of $0.25\mu\text{c}/\mu\text{g}$, and shortly afterwards Heinle et al. (1952) used this to measure the absorption of the vitamin in two control subjects and four persons with pernicious anaemia. Four separate isotopes-- ^{60}Co , ^{58}Co , ^{57}Co , and ^{56}Co --have been developed and used for measuring vitamin B12 absorption. ^{60}Co is no longer used because

TABLE I: *Vitamin B12 Analogues*

-(5,6 dimethylbenziminazolyl) cobamide cyanide -

CYANOCOBALAMIN

-(5,6 dimethylbenziminazolyl) aquocobamide -

AQUOCOBALAMIN

-(5,6 dimethylbenziminazolyl) hydroxocobamide -

HYDROXOCOBALAMIN

-(5,6 dimethylbenziminazolyl) cobamide nitrite -

NITRITOCOBALAMIN

-(5,6 dimethylbenziminazolyl)-Co-5'-deoxyadenosylcobalamide -

COENZYME B12/
DEOXYADENOSYLCOBALAMIN

-(5,6 dimethylbenziminazolyl)-Co-methylcobamide -

METHYLCOBALAMIN

of its very long half-life (1,290 days), and ^{56}Co has fallen into disuse, as the radiation dosage resulting from its use is high despite its relatively short half-life (77 days). ^{58}Co (half-life 71 days) and ^{57}Co (half-life 270 days) are now the isotopes of choice for human studies; subjects receive a relatively low radiation dose from ^{57}Co , despite its somewhat long half-life, because it has a low dose rate constant of gamma emission. Table II shows the radiation doses to various organs from the four isotopes (Reizenstein 1959), and Fig. 1 the scintillation spectra of the two most commonly used isotopes, ^{58}Co and ^{57}Co (Armstrong and Woodliff 1966). These two isotopes were used in the studies described below.

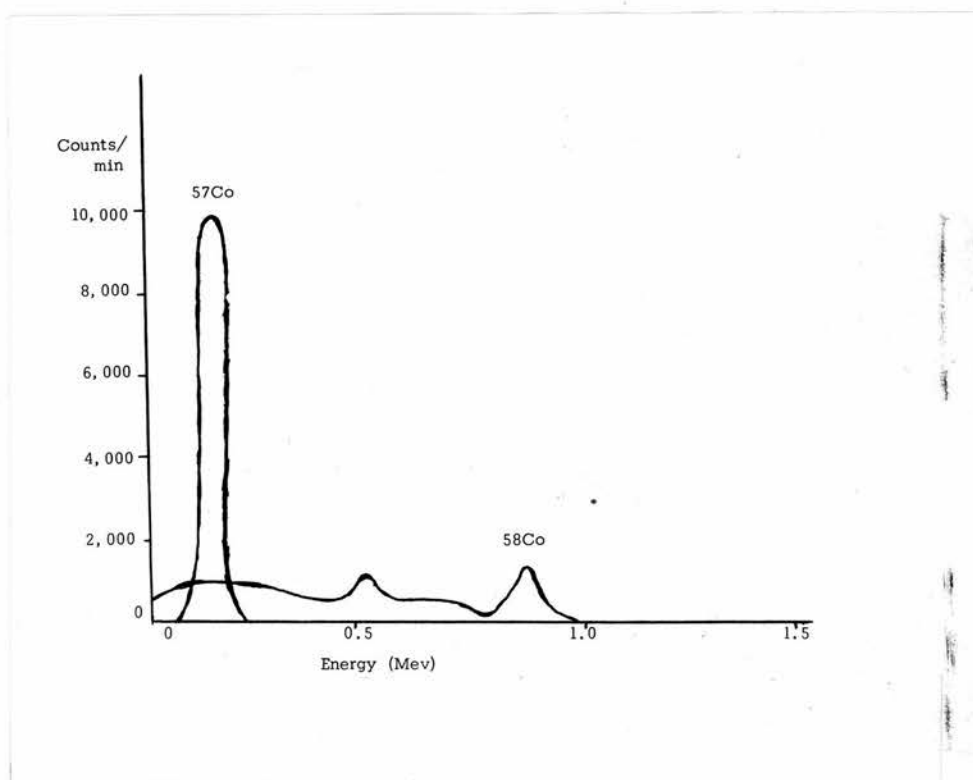
Dietary Content of Vitamin B12: Generally speaking, vitamin B12, which does not occur in plants, is synthesised by bacteria, all the vitamin B12 in higher animals deriving directly or indirectly from this source. Though there is considerable synthesis of vitamin B12 by large bowel bacteria, this is of no nutritional value in man, where large amounts, quantitatively similar to that found in normal persons, may be found in the faeces of patients with vitamin B12 deficiency due to the fish tapeworm (Kaipainen and Tötterman 1954) or to pernicious anaemia (Berk et al. 1948, Bethell et al. 1948, Kaipainen et al. 1954). Man is thus entirely dependent on a dietary intake of vitamin B12, the main sources of which are liver, kidney, meats of all kinds, and dairy produce (McCance and Widdowson 1960).

TABLE II: *Radiation Dose in Different Organs
from 1.0 μ c in 1.0 μ g of Vitamin B12
administered parenterally*

Organ	Radiation Dose (millirads)			
	60Co	58Co	57Co	56Co
Liver	2910	265	105	1465
Bone Marrow	730	60	28	300
Ovaries	245	25	12	120
Testes	600	55	14	185

(From: Reizenstein, 1959)

FIG. 1: Scintillation spectra of ^{57}Co and ^{58}Co



However, although it is possible to eat a vegetarian diet deficient in vitamin B12, so widespread is the vitamin that it is rare for gross deficiency states to occur for dietary reasons. Chung et al. (1961) have shown that although the vitamin B12 content of diets of otherwise widely varying quality differed greatly, even diets classified as of poor general quality probably contained sufficient vitamin B12 (mean 2.7µg/day) to prevent deficiency syndromes even after cooking. In addition to the wide distribution of the vitamin, however, deficiency is also minimized by the relative lack of effect of cooking on the vitamin (Banerjee and Chatterjea 1963) compared, for example, to folic acid, and by the likelihood that almost all vitamin B12 ingested is equally available for absorption (Heyssel et al. 1966).

Human Vitamin B12 Balance: Many estimates have been made of the total vitamin B12 content of the human body, either by microbiological assay of organs or by isotope dilution methods. These estimates have given values ranging from a mean of about 5mg (Ross and Mollin 1957) to one of about 2mg (Adams 1962). Vitamin B12 intake from the diet varies greatly (Chung et al. 1961), as mentioned above, and ranges from about 85µg daily on a high quality diet to 1µg on a poor one. However, Heyssel et al. (1966) estimate that most normal Western adults probably consume 5 to 15µg daily of which 5µg at least is absorbed. This daily absorption of vitamin B12 in normal persons fits well with estimates of the daily obligatory losses of the vitamin, probably mainly in the urine and stools (Hall 1964), which amount to

about 0.1% of the total body store daily (Heyssel et al. 1966). As the daily obligatory vitamin loss varies with the total body store (Reizenstein 1959), it has been suggested that the minimal daily intake needed to maintain health in the absence of factors leading to increased demand, such as growth and pregnancy, is 0.6-1.2 μ g/day (Heyssel et al. 1966).

VITAMIN B12 ABSORPTION

It is generally recognised that vitamin B12 can be absorbed in two ways: first by a process of diffusion across the small intestinal wall, and second through a mechanism mediated by the gastric product, intrinsic factor, in which the absorption of the vitamin in the small intestine is limited to the terminal ileum. Only the intrinsic factor mediated mechanism will be considered, as the diffusion mechanism does not occur under normal circumstances.

INTRINSIC FACTOR

In a now classical series of papers (Castle 1929, Castle and Townsend 1929, Castle et al. 1930), Castle and his co-workers at Harvard University discovered the existence of "intrinsic factor" and laid the groundwork which has led to the present voluminous literature on vitamin B12 and its absorption. These discoveries were made in patients with pernicious anaemia who by 1929 were known to have atrophy of the gastric glands and to have gastric juices incapable of digesting protein (Fenwick 1870) and who had been shown to respond therapeutically to raw liver (Minot and Murphy 1926). The

Harvard workers showed that marked improvement in pernicious anaemia could be obtained by feeding patients beef muscle which had been incubated with normal gastric juice, though neither alone could achieve this effect. They showed that the effect could not be mimicked by hydrochloric acid or commercial pig pepsin, was not dependent on an acid pH, and could be destroyed by heating. Finally they demonstrated that only normal gastric juice or gastric mucosa possessed what they called the "intrinsic factor", needed to allow the absorption into the body of the "extrinsic factor" in beef muscle, now known to be vitamin B12. "Intrinsic factor" was not present in saliva or duodenal contents, where the latter were free of contamination with gastric juice, nor was it present in the gastric juice of patients with pernicious anaemia.

Vitamin B12 Binders in Gastric Juice: The binding of vitamin B12 is recognised as one of the fundamental properties of the intrinsic factor in gastric juice. However, it is known that vitamin B12 binders other than intrinsic factor are present in gastric juice, and these will not promote vitamin B12 absorption. The nature of all the vitamin B12 binders in gastric juice has been investigated predominantly by the groups of Grasbeck (1962) and of Glass (1963). There are three vitamin B12 binders in gastric juice, and these have received various designations. In pooled gastric juice collected after betazole stimulation Simons (1964) found that intrinsic factor (binder S, primary binder) constituted 63% of the

binders, altered intrinsic factor (binder I, secondary binder) 19%, and non-intrinsic factor binder (binder R, tertiary binder) 18%. The relative amount of non-intrinsic factor binder is higher in fasting gastric juice and falls when gastric secretion occurs. Gullberg and Olnagen (1959) and Simons (1964) showed that the altered intrinsic factor binder could be eliminated by in vivo neutralisation of gastric juice, and it has also been shown that this binder is absent from achlorhydric gastric juice (Glass et al. 1962, Simons 1964); it is probably produced by the action of pepsin on intrinsic factor.

There are, therefore, basically two vitamin B12 binders in gastric juice: intrinsic factor and non-intrinsic factor binder. A very important practical implication of this is that gastric juice vitamin B12 binding capacity cannot be equated with its intrinsic factor content. In this regard, it is pertinent to note that intrinsic factor antibodies derived from persons with pernicious anaemia do not react with non-intrinsic factor binder. The non-intrinsic factor binder will, however, react with antisera to the vitamin B12 binder found in saliva (Simons 1964).

Isolation of Human Intrinsic Factor: Human intrinsic factor has been isolated in greatest purity by Grasbeck et al. (1966). This was achieved, with 40 liters of pepsin-inactivated gastric juice, using the methods of ultrafiltration for concentration followed by a variety of chromatographic techniques. Analysis, by ultracentrifugation and by electrophoresis, of the intrinsic factor (binder S) fraction showed it to be homogeneous, to have a molecular weight of

119,000, to be a glycoprotein, and to be able to bind 25 μ g of vitamin B12 per mg of protein. This latter fact suggested that two molecules of vitamin B12 were being bound per molecule of intrinsic factor. However, as previous work had suggested that intrinsic factor had a molecular weight of 60,000, the authors suggested that their material was in fact an intrinsic factor dimer. Thus one molecule of intrinsic factor would bind one molecule of vitamin B12.

Physicochemical Characteristics of Intrinsic Factor: Intrinsic factor is known to have a number of properties which are important in relation to its biological activity and which it is important to be aware of in work involving its estimation. Castle et al. (1937) originally observed the thermolability of intrinsic factor after exposure to 100°C for five minutes, and since then it has been shown that thermolability occurs at even lower temperatures. Intrinsic factor activity has been found to be stable between pH 2.0 and pH 10.0 (Grasbeck 1956), though activity is rapidly lost above pH 10.0 (Castro-Curel and Glass 1963). Intrinsic factor is also destroyed by pepsin (Grasbeck 1956), and the vitamin B12 binding capacity of acid gastric juice falls steadily, this being much less at room temperature than at 37°C (Abels and Schilling 1964). The molecule, however, seems to resist the action of a number of other enzymes, including trypsin, papain, and tyrosinase.

In all of the work cited above, intrinsic factor activity was measured either in vivo or in vitro by its ability to promote vitamin B12 uptake by ileal preparations. In 1963 the radioimmunoassay for

intrinsic factor was introduced (Ardeman and Chanarin 1963). This assay has been very widely used since then and was used in the work described below. There have been very few reports on the effects of factors such as temperature, pH, and pepsin on immunoassayable intrinsic factor, and none of it was available when this work was started. McGuigan (1967) has since shown that the affinity constant of vitamin B12 for intrinsic factor is very high and does not alter between pH 4.5 and pH 11.0 in depepsinized gastric juice. According to Irvine (1966), the intrinsic factor content of normal human gastric juice is unaltered by a pH of 2.0 for two hours at room temperature but is destroyed after thirty minutes at 56°C. The former finding is in contrast to that of Ashworth et al. (1969), who found a prompt and marked reduction in intrinsic factor content in acidic gastric juice; these workers provided evidence that the loss was due to the action of pepsin. The present author's experience is based on experimental work described later, but in summary, while intrinsic factor was stable at pH levels from 2.1 to 10.0 and at temperatures from 4°C to 37°C in depepsinized gastric juice, a reduction of pH in the presence of pepsin at 37°C resulted in a prompt loss of intrinsic factor.

Production and Secretion of Intrinsic Factor: Until recently it was known only that intrinsic factor was produced in the fundus of the human stomach. This was based on the observations that atrophic gastritis in pernicious anaemia was confined to the fundus (Magnus and Ungley 1938), the normality of the antrum in that condition being

confirmed by recent work on gastrin secretion (Strickland et al. 1971), that proximal gastrectomy gave rise to vitamin B12 malabsorption almost as often as total gastrectomy, which will always produce malabsorption, and that only preparations from the fundic part of the stomach will promote vitamin B12 absorption efficiency in pernicious anaemia (Posth et al. 1962). In 1964, however, Hoedemaeker et al. demonstrated by immunoautoradiographic methods that in man the parietal cell is the source of intrinsic factor. As the parietal cell is also the source of hydrochlorhydric acid, this discovery led to many studies of the secretion of intrinsic factor, particularly in relation to acid secretion and in response to agents known to stimulate acid secretion. In general, it has been found that men secrete more intrinsic factor than women both in the basal state and after gastric secretory stimulation, and that this is solely the result of the greater gastric juice volume produced by the former, intrinsic factor concentrations being the same (Ardeman et al. 1965). In older persons over the age of 50 years intrinsic factor secretion falls. It has been shown that there is a considerable secretion of intrinsic factor even in the basal state (Ardeman et al. 1964) with average hourly outputs of 3000ng units, and that any of those substances known to be potent stimulators of acid secretion, such as histamine (Ardeman et al. 1964, Rødbro et al. 1965), histalog (Ardeman and Chanarin 1965) gastrin (Wangel and Callender 1965, Irvine et al. 1965), pentagastrin (Shearman et al. 1967, Irvine et al. 1968), and insulin (Ardeman et al. 1964), will cause a marked increase in intrinsic factor secretion. The

absolute amounts of intrinsic factor secreted after stimulation vary, and much of the variation can probably be attributed to the varying completeness of gastric juice collections in different reports. The extent to which different gastric stimulants give comparable results on intrinsic factor secretion has been much less studied. In the work described below, two different powerful stimulants, histamine and pentagastrin, were used for reasons detailed later. It had previously been shown by others that histamine (0.04mg/kg of body weight) and pentagastrin (6µg/kg of body weight) gave comparable acid secretory responses (Makhlouf et al. 1966, Wormsley et al. 1966, Multicenter Pilot Study 1967, Kontureck 1967), and the studies of the author and his colleagues showed that in these doses the two agents had closely similar effects on intrinsic factor secretion (Shearman et al. 1967). These studies also illustrated the very significant correlation known to exist between acid and intrinsic factor secretion (Ardeman 1965, Rødbro^{et al.} 1965), and showed the different secretion patterns for the two substances (see Figs. 11 and 12 in "Material and Methods") when continuous gastric secretory stimulation is given (Dotevall et al. 1967). These patterns suggest that whereas acid is produced only in response to stimulation, intrinsic factor is stored and gives a "wash out" pattern on stimulation.

Assay of Intrinsic Factor: Intrinsic factor may be assayed either in vivo or in vitro. The in vivo methods, which depend on demonstrating that a material will promote vitamin B12 absorption in

a subject with pernicious anaemia, are cumbersome and time consuming but have the advantage of demonstrating capacity to cause actual absorption. They do not give quantitative results. The in vitro radioimmunoassay is by far the best method currently available for measuring human intrinsic factor, and the most accepted simple method giving reproducible results is that of Ardeman and Chanarin (1963). This assay, which was used in the work described below, depends on the use of specific antibody to intrinsic factor and the vitamin B12 absorbent charcoal.

INTRINSIC FACTOR-VITAMIN B12 INTERACTION

As described above, Grasbeck et al. (1966) have suggested that each molecule (monomeric) of intrinsic factor binds one molecule of vitamin B12, and McGuigan (1967) has demonstrated its great avidity for the vitamin. The union of intrinsic factor and vitamin B12 occurs very quickly and as the molecule becomes smaller, as defined by gel filtration and sedimentation studies, after binding to vitamin B12, Grasbeck (1967) has suggested that the intrinsic factor molecule folds round the vitamin B12 molecule. The nucleotide and the corrin ring of the vitamin B12 molecule seem to be of greatest importance in the union with intrinsic factor, the ligand being of relatively little importance. It has been shown that exchange between bound and unbound vitamin B12 occurs both in vivo and in vitro, and in vitro this is temperature dependent. Once bound to vitamin B12, human intrinsic factor becomes more resistant to the destructive effects of heat and pepsin (Abels and Schilling 1964). On the other hand, the binding of

vitamin B12 to intrinsic factor may prevent the uptake of the former by some of the bacteria likely to be found in blind-loop syndrome (Donaldson et al. 1962).

ILEAL ABSORPTION OF VITAMIN B12

It is now fully accepted that in humans, physiological vitamin B12 absorption is restricted to the terminal two to six feet of the ileum. For normal absorption to occur, however, not only must the ileal epithelial cells be healthy, but the luminal small intestinal environment must also be normal. Disturbances in the latter, such as excess acidity, as in the Zollinger-Ellison syndrome, lack of pancreatic exocrine secretion, or the excessive local proliferation of bacteria, may cause this. In this review of normal absorption, however, these factors will not be considered further.

When an oral dose of radioactive vitamin B12 is given, detectable vitamin B12 does not appear in the blood for four hours and does not reach a peak for some eight to twelve hours (Booth and Mollin 1956). It has been found that this long delay is due to the time needed for the vitamin B12 to pass from the epithelial cell surface into the body. Much of current research centers on the mechanism of this absorption, which is still largely unknown. That there must be a specific mucosal receptor site is indicated by the anatomical restrictions of the absorption area and the ease with which the intrinsic factor absorption mechanism can be saturated. Donaldson et

al. (1967) demonstrated that in hamsters the specific receptors may be in the microvillous membranes of the villous epithelial cells, and later work from the same group (MacKenzie et al. 1968) showed that these sites could be blocked by antibody to distal but not to proximal epithelial microvillous membranes. In humans it has been shown that only ileal homogenates will show an enhanced intrinsic factor-vitamin B12 uptake which can be blocked by intrinsic factor antibody (Carmel et al. 1969). Although uptake of the intrinsic factor-vitamin B12 complex may not require a source of energy, the available evidence suggests that the presence of calcium or magnesium ions is needed and that the process occurs best at pH 6.6 (Cooper and Castle 1960, Carmel et al. 1969).

It is currently considered that the transfer of vitamin B12 across the ileal cell requires that it be separated from the intrinsic factor, as the latter has not been identified in association with absorbed vitamin B12 reaching the intestinal lymphatics in rats (Boass and Wilson 1964) or the portal blood in man (Cooper and White 1968). The work of Peters and Hoffbrand (1970) in guinea pigs using ^{125}I -labelled intrinsic factor and ^{57}Co vitamin B12 support this idea, as only the vitamin B12 entered the cell. The separation of the complex preceded the period of delay in absorption, and it has been supposed that separation may be effected by brush border hydrolase enzymes (MacKenzie and Donaldson 1969). Peters and Hoffbrand (1971) also showed that the vitamin B12 passes

to the mitochondria before entering the body. What happens to the vitamin B12 in the ileal epithelial cell is not known, but it eventually enters the blood attached to a carrier protein, transcobalamin II (Hall and Finkler 1965). This carrier has a very short half-life (Hall 1969), so that the vitamin B12 quickly reaches the tissues.

BODY DISTRIBUTION OF VITAMIN B12

Absorbed radioactive vitamin B12 enters the plasma bound to a β globulin, transcobalamin II (Hall and Finkler 1965). This has a very short half-life, and there is evidence that transcobalamin II, which is probably produced by the liver (Haught et al. 1967), may promote vitamin B12 uptake by cells (Finkler and Hall 1967). Little of the vitamin reaches the urine (see "Results" below). Later, vitamin B12 re-enters the plasma bound to an α globulin, transcobalamin I (Hall and Finkler 1965), which does not promote cell uptake of the vitamin (Finkler and Hall 1967); it has been suggested that transcobalamin I is a cell-exit vitamin B12 carrier (Finkler and Hall 1967). Over the first week after absorption there is a rapid rise in hepatic radioactivity followed by a more gradual rise due to a slow but continuous further transfer to the liver. Final equilibration from a single oral dose may take about one month (Bozian et al. 1963).

Absorbed vitamin B12 is re-excreted in the bile, and this appears to be the major source of excretion from the body (Ardeman et al. 1965). Relatively little of this biliary excretion of vitamin B12 reaches the faeces, however, some three quarters being reabsorbed

(Grasbeck et al. 1958). Vitamin B12 excreted in bile requires intrinsic factor for its reabsorption (Booth and Spray 1960).

LIMITS OF VITAMIN B12 ABSORPTION

There is a limit to the amount of vitamin B12 which can be absorbed from a single oral dose, and beyond the limit there is a refractory state to further uptake. This refractory state probably lasts for less than 4 hours (Heyssel et al. 1966). It has been suggested (Swenseid et al. 1954) that the mean maximum absorption for any one dose is about 1.6 μ g, though individuals may absorb up to 3.0 μ g. Swenseid et al. (1954) found that some subjects, especially those who absorbed relatively little in the fasting state, absorbed more when the test dose was given with a vitamin B12-free meal. This has been found to occur more often in persons following partial gastrectomy (Deller et al. 1961, Turnbull 1967). On the other hand, Heyssel et al. (1966) found that natural dietary vitamin B12 and an aqueous solution of vitamin B12 were equally well absorbed in normal persons. These last investigators suggested that vitamin B12 absorption may diminish with age (Heyssel et al. 1966).

It has been estimated that 400-500ng units of intrinsic factor are needed to allow normal absorption from 1 μ g of vitamin B12, though more may be required after gastric surgery (Ardeman and Chanarin 1965).

SUMMARY

Vitamin B12 and its analogues are a group of substances which comprise a corrin ring, a nucleotide, and a ligand the nature of which

varies. Deoxyadenosylcobalamin is the most important form occurring in nature, where all vitamin B12 derives from bacterial synthesis. The main sources of vitamin B12 are animal protein products, and the daily minimal requirement for the vitamin in humans is about 0.6-1.2 μ g.

Normal absorption of vitamin B12 requires the production of intrinsic factor, which is secreted simultaneously with acid by the gastric fundal parietal cells in man. Intrinsic factor is a thermolabile glycoprotein, susceptible to the proteolytic action of pepsin, which will bind one molecule of vitamin B12 per molecule of intrinsic factor and promote its absorption from the intestine. This latter property distinguishes intrinsic factor from other vitamin B12 binders produced by the stomach.

Vitamin B12 is absorbed solely in the terminal ileum in man, and specific receptor sites there seem to determine this function. The uptake of the vitamin at any given time is limited, and many abnormalities of the intestinal luminal environment may interfere with this. In the terminal ileum, vitamin B12 seems to be separated from intrinsic factor at the epithelial cell surface, only the vitamin B12 entering the cell. A number of unknown metabolic processes occur within the ileal epithelial cell, perhaps in the mitochondria, before the vitamin B12 enters the body, where it is bound to a plasma protein, transcobalamin II, which promotes its uptake by tissues.

It appears that most forms of vitamin B12 are well absorbed, and food vitamin B12 is absorbed as well as aqueous cyanocobalamin.

DEFINITION OF PERNICIOUS ANAEMIA

The definition of pernicious anaemia is central to a close consideration of the results obtained with any test designed to measure vitamin B12 absorption. The reason for this is that pernicious anaemia is one disease in which severe malabsorption of the vitamin should always be found by definition. In other conditions such as partial gastrectomy, coeliac syndrome and Crohn's disease, severe vitamin B12 malabsorption may occur, but is by no means universal. Consequently, the basic first assessment of a vitamin B12 absorption test is to see whether it can differentiate between subjects without gastrointestinal disease and those with pernicious anaemia. The only other condition which can replace pernicious anaemia for such a purpose is where a subject has had a total gastrectomy; this, however, is an operation which is rarely done, and no such subjects were available for the study described below.

The term pernicious anaemia is frequently used incorrectly to refer to vitamin B12 deficiency megaloblastic anaemias following partial gastrectomy or due to congenital ileal vitamin B12 malabsorption (Grasbeck et al. 1960, Imerslund 1960). In fact, it may be defined as vitamin B12 deficiency resulting from malabsorption of vitamin B12 owing to absent, inadequate or ineffective intrinsic factor secretion by the intact stomach. The question as to what is inadequate intrinsic factor secretion in quantitative terms is the subject of a dispute which is discussed below. However, in this

study subjects were accepted as having pernicious anaemia if they had intact stomachs and malabsorption of vitamin B12 which could be corrected fully with exogenous intrinsic factor as assessed by the Schilling test (see "Materials and Methods"). Phenomena such as parietal cell antibodies and intrinsic factor antibodies which occur in pernicious anaemia were looked for, but as they are not central to the diagnosis, they were not taken into account in this regard.

METHODS FOR THE MEASUREMENT OF VITAMIN B12 ABSORPTION

All generally accepted methods for measuring vitamin B12 absorption at the present time depend on the use of radioactive isotopes, and these assays "compare with the hematological assays of the past as a 1963 leisurely motor drive from Marathon to Athens compares with the Marathon run of the old Greeks" (Glass 1963). At the present time five different isotope methods have been developed for this purpose, and these depend on the measurement of faecal, urinary, plasma, hepatic or whole body radioactivity, under specific circumstances, after the administration of test doses of the isotopically labeled vitamin. In this section, the advantages and disadvantages of the first four methods will be reviewed, after which the principles of whole body counting and the work done so far on their application to measuring vitamin B12 absorption will be stated.

Faecal Excretion Test.

Heinle et al. (1952), who were the first to demonstrate intrinsic factor dependent malabsorption of vitamin B12 in pernicious

anaemia, did so using this test. They found that when given 0.5 μ g of ^{60}Co vitamin B12, three persons with pernicious anaemia absorbed 4-28% of the test dose, and that this could be raised to 71-95% with added hog intrinsic factor; two control subjects absorbed 58-100% of this test dose.

The principle of the test is to give a test dose of vitamin B12 orally, collect the faeces until excretion of radioactivity ceases, and derive the amount absorbed from that found in the faeces. It is assumed that faecal radioactivity represents excreted vitamin B12, that this excreted vitamin B12 was not absorbed, and that the difference between ingested and excreted radioactivity represents that amount of vitamin B12 absorbed into the body. As Glass (1963) has stated, there is the possibility that some faecal radioactivity may represent vitamin B12 re-excreted in the enterohepatic circulation and not reabsorbed, and that unabsorbed radioactivity may be trapped for a considerable time in, for example, diverticula and be counted as absorbed. Most workers agree that excreted radioactivity truly represents vitamin B12 and accept that this is a valid test method.

The test has three main advantages: it is potentially quantitative; no parenteral vitamin B12 is given, so that metabolic and haematologic changes resulting from this do not occur; and it is not invalidated by renal failure. On the other hand, the test takes a long time to perform, is aesthetically distasteful to most subjects, resulting in frequently poor faecal collections (see "Discussion")

below), and has a poor reproducibility with a wide normal absorption range which may overlap with the malabsorption range (Glass 1963).

The results obtained in the faecal excretion test depend on the dose of vitamin B12 given. Very variable doses have been used by different workers, and this has been criticised by Grasbeck (1962). For example, the work of a variety of centers showed that the calculated normal absorption from a 0.5 μ g dose was 20-95%, from a 1.0 μ g dose 26-88%, and from a 2.0 μ g dose 4-83% (Glass 1963). Where workers have used a 0.5 μ g dose, as in the work described below, the range of absorption in pernicious anaemia has been 0-28% as compared to 20-95% in controls (Heinle et al. 1952, Callender et al. 1954, Halsted et al. 1956). When intrinsic factor was given to the pernicious anaemia patients in these series, the absorption range rose to 34-95%. It can be seen that there is some overlap in these ranges.

In 1965 Ganatra et al. introduced a double isotope method in an attempt to simplify the faecal excretion test while retaining it as a quantitative test of absorption. They used the nonabsorbable gastrointestinal marker 51Cr (Whitby and Lang 1959) as chromic oxide and showed that the percentage absorption from a dose of 58Co vitamin B12 could be calculated from single stool specimens taken within the first 48 hours after both isotopes had been given together. This was done from the formula:

$$\text{Absorption (\%)} = 100 - \frac{100 (51\text{Cr in standard} \times 58\text{Co in stool})}{51\text{Cr in stool} \times 58\text{Co in standard}}$$

Moreover, single stool specimens collected within the 48 hour period gave closely similar results. Mollin and Walters (1967) were unable to reproduce the promising results of Ganatra et al. (1965), but more recently good results have been reported by Campbell and Craswell (1970). These last workers used fluid 51Cr chromic chloride as the marker and suggested that this may give better results than insoluble 51Cr chromic oxide, the gut dispersal of which probably differs from that of fluid 58Co vitamin B12.

In summary, most workers agree that the faecal excretion test is a valid way of measuring vitamin B12 absorption quantitatively, albeit indirectly. The main drawback is the high incidence of incomplete collections of faeces due to the unaesthetic nature of the test, though this problem may be made easier by the use of double isotope methods.

Urinary Excretion Test.

The urinary excretion test depends on the observation that the majority of a large dose of nonradioactive vitamin B12 passes rapidly into the urine, 80% of it being excreted within 24 hours. During this period it carries with it a substantial part of any radioactive vitamin B12 absorbed simultaneously into the blood from the gut. It has been shown that the radioactivity in the urine truly represents vitamin B12 (MacLean and Bloch 1954), that about a third of the radio-

activity absorbed in normal persons is carried into the urine in the 24 hour period (Callender and Evans 1955, Cottrall et al. 1971), though this is less where there is malabsorption, and that the peak excretion of radioactivity occurs between the sixth and twelfth hours.

This test was originally described by Schilling (1953), and in its original form a test dose of 2.0 μ g was given with the so-called "flushing" dose of unlabelled vitamin B12 being given intramuscularly two hours later. Urine was collected over a 24 hour period and the excreted radioactivity expressed as a percentage of that given orally. Since 1953 this test, which has become the most widely used one in clinical practice, has been variously modified as regards the oral test dose, the interval between giving the oral test dose and the nonradioactive "flushing" dose, the number of "flushing" doses, the duration of the urine collection, and the way of counting urine radioactivity. The results of all these modifications can be summarised by saying that higher percentage excretions of radioactivity are obtained by lowering the amount of the oral test dose, increasing the number of "flushing" doses, and prolonging the urine collection time over that stated above. Glass (1963) has illustrated this by summarising the reports of various workers. Where a test dose of 2.0 μ g of radioactive vitamin B12, one "flushing" dose of 1.0mg of nonradioactive vitamin B12, and a 24 hour urine collection period were used, normal excretion ranged from 10% to 16%; when

the respective criteria used were a test dose of less than 1.0 μ g, one "flushing" dose, and a 24 hour collection period, normal excretion was 15-26%; with two "flushing" doses and 48-72 hours of urine collection, normal excretion was 30-37% from a test dose of less than 1.0 μ g and 13-18% from a 2.0 μ g test dose. Even when 72 hour excretion values are used, patients with pernicious anaemia usually excrete only up to 8% of the test dose, with mean values of 0.4-3.8%. More than one "flushing" dose does not increase excretion of radioactivity in pernicious anaemia, and overlap between pernicious anaemia and normal subjects is uncommon whatever methodological variant is used. When potent human or hog intrinsic factor was added to the test, this markedly increased, but did not always rectify completely, absorption in pernicious anaemia. These results have been confirmed by more recent workers (Lamar et al. 1965).

Relatively little work has been done on the reproducibility in the same subject of any of the vitamin B₁₂ tests described. It is therefore important to note that Adams and Cartwright (1963) have questioned the validity of single urinary excretion tests, at least in patients with intestinal malabsorption or in those who have had partial gastrectomy. They found consistently normal or subnormal values in only one of seven malabsorptive patients and in only two of eight patients with partial gastrectomy. The reasons for the variability were not determined, but the importance of their findings is clear.

Katz et al. (1963) modified the urinary excretion test by introducing a double isotope test for the detection of intrinsic factor mediated vitamin B12 malabsorption. These workers, who gave ^{60}Co vitamin B12 bound to normal human gastric juice and free ^{57}Co vitamin B12 orally at the same time, were able to show a clear separation between the ratios of the isotopically labeled vitamin which had been bound to intrinsic factor and that which had not in the 24 hour urine specimen in controls (0.97: range 0.83 - 1.16) and patients with pernicious anaemia (2.60: range 1.61 - 3.63). This work was confirmed by Bell and Lee (1969), using ^{57}Co and ^{58}Co , who also showed that circulating serum intrinsic factor antibodies in six pernicious anaemia patients did not seem to inhibit the absorption of the intrinsic factor bound ^{57}Co vitamin B12. Although Glass (1963) suggested that exchange of the bound and free isotopes in the gut might invalidate this form of the test, in practice this does not seem to have happened.

The clinical value of this test is universally acknowledged, and this is reflected in its being the test which is most widely used. It is simple from the clinical and laboratory viewpoints, relatively rapidly done, and at least in pernicious anaemia seems consistently to differentiate between controls and persons with malabsorption. The flushing dose also removes about a third of the absorbed radioactivity in persons with normal absorption. On the other hand, from a research point of view it has the drawbacks of being only qualitative or at most

semiquantitative, of needing subjects who can co-operate in urine collection, and of altering vitamin B12 metabolism and the haematological picture as a result of giving the "flushing" vitamin B12 dose. It may also underestimate vitamin B12 absorption, as some of the "flushing" dose may be excreted in the bile into the gut, where it could reduce the absorption of intrinsic factor bound radioactive vitamin B12 by exchanging with the latter. Finally, ^{et al.} the test is invalid in the presence of renal impairment (Rath 1957).

Hepatic Uptake Test.

This test, originally described by Glass et al. (1954) is based on the knowledge that a large proportion of an oral test dose of radioactive vitamin B12 absorbed from the intestine is deposited in the liver. Surface scintillation counting of radioactivity is carried out one week after the administration of the oral test dose to allow time for the excretion of unabsorbed radioactivity, and to allow peak accumulation of radioactivity in the liver to occur. The test can be done two days after the oral dose is given if unabsorbed radioactivity is actively removed from the bowel (Glass and Boyd 1957). Counts are carried out over two or three liver projections, and corrected for background (obtained by a calf count) and decay of activity. Attempts are made to detect unabsorbed radioactivity by counting over the left iliac fossa, count rates in this region exceeding 50% of the liver count rate indicating unabsorbed and unexcreted material.

Many modifications have been made to the original test, including the use of large scintillation crystals (Johnson et al. 1958), very high specific activity ^{56}Co vitamin B12 (Booth and Mollin 1956), and the use of single projection counts (Pollycove and Apt 1956). All of these methods seem to give satisfactory results for routine clinical purposes, and there is virtually no overlapping between control subjects and those with pernicious anaemia or total gastrectomy. Typical results are quoted by Glass (1963) in his review of this topic. Using a $0.5\mu\text{g}$ test dose of vitamin B12, healthy persons had liver counts of 200-5300 counts/min/ μc of the oral dose, pernicious anaemia patients 0-769 counts/min/ μc , and after intrinsic factor the counts in the latter group rose to 120-3587 counts/min/ μc .

This test has many advantages when looked at from the clinical viewpoint. It does not require urine or stool collections, the taking of blood is unnecessary, and renal disease does not interfere with it. No "flushing" doses of vitamin B12 are given and so subsequent patient investigations are not made void. It is simple, and results generally clearly separate those with normal absorption from those with pernicious anaemia. On the other hand, owing to difficulties in establishing baselines consequent on the accumulation of radioactivity in the liver, repeated absorption tests with this method may be difficult. Furthermore, coexistent liver disease may decrease the amount of radioactivity in that organ (Glass et al. 1958). This impaired uptake cannot be reversed by intrinsic factor. Finally,

Townsend et al. (1968) have commented on the poor reproducibility of this test and on the wide range of normal absorption.

More recently, Weisberg and Glass (1966) have reported on their efforts to make the hepatic uptake quantitative. This was done by using a double isotope method in which 0.5 μ g of ^{60}Co vitamin B12 was given orally and 0.1 μ g of ^{57}Co vitamin B12 was given intravenously. The percent absorption of the oral dose was calculated from the formula

$$\frac{\text{oral dose hepatic counts}}{\text{intravenous dose hepatic counts}} \times 100$$

The method assumes that the tissue distribution of orally and intravenously administered vitamin B12 is the same, for which there is evidence (Reizenstein, 1963); and correction factors have to be introduced to allow for the different counting efficiencies of ^{60}Co and ^{57}Co . The authors consider the method is quantitative and claim that, as ratios are used, it eliminates the difficulties of varying liver geometry in different subjects and the problem of poor liver function in those with significant liver disease.

Plasma Radioactivity Test.

This test, though in no way quantitative, has the advantages of simplicity: there is no need for urine or faecal collections and a result is obtained in less than 24 hours. Similar curves for plasma radioactivity have been obtained by all workers, and it is now recognised that for clinical purposes a single 8 hour plasma sample is all

that is needed. The test can be done without giving "flushing" doses of vitamin B12, though Coupland (1966) and others have shown that this greatly increased plasma activity levels. The test can be used in the presence of renal failure.

At first the test did not gain wide acceptance in spite of its simplicity, because relatively large amounts of then-used isotopes such as ^{56}Co (Booth and Mollin 1956) and ^{60}Co (Doscherholmen and Hagen 1957) were needed to produce rather low plasma activity levels. With the introduction of ^{57}Co , however, good plasma radioactivity could be obtained with relatively small oral test doses, and the diagnostic discriminatory power of the test was improved by the discovery that plasma radioactivity was increased by "flushing" doses of vitamin B12 (Coupland 1966). Since then a number of reports have substantiated the claim that where ^{57}Co vitamin B12 is used, the test has good diagnostic value (Workman and Rusche 1966, Forshaw and Harwood 1966, Finney and Payne 1968, Arkun et al. 1969), though occasional unusual results are obtained (McIntyre and Wagner 1966, Coiner and Walsh 1971).

Correlation Between Tests.

Several workers have reported on the interrelation of results obtained using more than one of the above tests on the same subjects. There is somewhat limited value in trying to obtain linear correlations between tests that are not both quantitative, such as the urinary excretion and plasma radioactivity tests; but in general it may be

said that in the majority of instances there is good agreement in the discrimination of normal absorption and malabsorption between these four tests (Callender and Evans 1955, Pollycove and Apt 1956, Woodliff and Armstrong 1966, Donaldson et al. 1970), though exceptions have been noted (McIntyre and Wagner 1966). An almost straight line relation has been reported between the hepatic uptake and faecal excretion tests (Pollycove and Apt 1956, Fone et al. 1961).

Summary.

Four methods of testing vitamin B12 absorption have been described: the faecal excretion method, the urine excretion method, the hepatic uptake method, and the plasma radioactivity method. All four give satisfactory results for clinical purposes under optimal conditions, though only the faecal excretion and perhaps the hepatic uptake method can be considered as quantitative. The faecal excretion and hepatic uptake tests are subject to errors from counting unabsorbed and unexcreted radioactivity; the faecal excretion and urine excretion tests require the accurate collection of body excretions, which is often faulty; the urine excretion and plasma radioactivity tests involve giving large "flushing" doses of parenteral vitamin B12, which alter body metabolic processes; and the plasma radioactivity test is wholly qualitative. There is on the whole a satisfactory correlation between the results obtained with these different tests.

VITAMIN B12 ABSORPTION TESTS WITH GASTRIC STIMULATION

In an attempt to improve the separation of absorption test results in persons who can produce sufficient intrinsic factor to effect vitamin B12 absorption from those in persons who cannot, a number of workers have given gastric stimulants when carrying out vitamin B12 absorption tests. The results of these efforts will be briefly described.

Carbachol.

Baker and Mollin (1955) originally reported that carbachol 0.25mg intramuscularly could increase the absorption of vitamin B12 in certain subjects with low absorption values. Some of these subjects had acid in the gastric juice and some did not, but none had pernicious anaemia. Mollin et al. (1957) confirmed these results and showed that carbachol, given at the time of a faecal excretion test, eliminated any overlap of results between pernicious anaemia patients and control subjects; this occurred as carbachol increased absorption only in the latter group. These workers suggested that the effect resulted from a stimulation of intrinsic factor secretion by carbachol; however, it has been shown more recently (Ardeman et al. 1964, Schipperijn 1965) that carbachol does not cause secretion of intrinsic factor. An alternative explanation is that carbachol produces its effect by increasing bowel motility, thus carrying the vitamin B12-intrinsic factor complex to the ileum before significant proteolytic inactivation has occurred. Whiteside et al. (1964) and

Siurala et al. (1960) have shown that carbachol may increase absorption in certain patients with atrophic gastritis. Dellipiani and Seaton (1965), on the other hand, found no increase in absorption in control subjects or postgastrectomy patients following the use of carbachol.

Histamine and Pentagastrin.

Kanaghinis et al. (1968) have claimed that 1.0mg of histamine phosphate given intramuscularly half an hour before a urinary excretion test will increase absorption to the same extent as carbachol. This certainly seemed to be the case in many instances, but the finding was far from universal. Irvine et al. (1970), on the other hand, saw no consistent effect of either histamine or pentagastrin on vitamin B12 absorption using the whole body counting method in subjects with or without pernicious anaemia. Armstrong and Woodliff (1967) saw no effect of carbachol or gastrin in their group of pernicious anaemia subjects.

Food.

Rune (1966) has shown that food is equivalent to histamine as a stimulus to gastric acid and therefore to intrinsic factor secretion. This is of interest in that Swenseid et al. (1954) using the faecal excretion test showed increased absorption of vitamin B12 in seven normal subjects when the test dose (0.5µg) was given with a meal. Deller et al. (1961) using the faecal excretion test were unable to show this effect in control subjects or in those with

pernicious anaemia, but found that food significantly improved absorption after partial gastrectomy, a finding confirmed by Turnbull (1967). Grasbeck et al. (1956) found that there was no effect of food on vitamin B12 absorption when the vitamin was given three hours after a meal.

In summary, carbachol seems to be able to increase vitamin B12 absorption in some subjects with low absorption values by an unknown mechanism. This does not occur in pernicious anaemia. Food, which stimulates gastric secretion, can also do this, especially after partial gastrectomy, and histamine or pentagastrin may do it occasionally in individual subjects.

WHOLE BODY COUNTERS

In this section the types of whole body counters available will be mentioned and the broad principles governing the use of that employed in this study described. Whole body counters, which are also known as low-level radiation counters, are instruments designed to detect minute amounts of radioactivity in human subjects. Currently there are two principal types available: liquid scintillation counters, which have high counting efficiency and sensitivity but limited gamma-ray energy resolution, and solid crystal counters, which are slower and less sensitive but have much higher resolution of gamma-ray spectra, permitting precise identification of different nuclides. Because of their inherent versatility, solid crystal counters have been widely accepted and are very suitable for the type of tracer studies reported here.

In general, whole body counters used in work with humans comprise a structure to hold the subject in position for counting, a detector to detect gamma radiation emitted by the subject, a mechanism for counting the radiation detected, and some means for excluding, or greatly reducing, extraneous (background) radiation.

The geometry of the position in which the subject is held for counting is of some importance, particularly as no one geometry is best under all circumstances. There are three commonly used geometries: the tilting chair, the arc bed (the arc generally being of one meter), and the scanning bed, in which the detectors

scan over the subject. In general, when redistribution of administered isotopes occurs in the body, counting rates vary considerably if the tilting chair geometry is used (Price et al. 1962, Naversten 1964), and this is particularly the case where the isotope tends to accumulate in certain organs, as is the case with vitamin B12. Naversten (1964), using phantoms, showed that a relative response of 0.8-2.0 for the same radioactivity concentrated in different sites could be reduced to 0.95-1.15 by a scanning system. The satisfactory performance of the scanning system, used in the present work, has been demonstrated for radioactive vitamin B12 in practice (Callender et al. 1966).

The whole body counter used in this study had thallium-activated sodium iodide crystals as detectors of radiation. When a gamma ray of the energy range utilized in medical applications (under 2.0 Mev) interacts with matter, it is usually by collision with an electron, and some or all of its energy is transferred to that electron. In certain materials, such as thallium-activated sodium iodide crystals, the energy reappears instantly as photons of visible or ultraviolet light, which can be detected and converted to electrical pulses by a photomultiplier tube. These crystals are generally hermetically encapsulated in aluminum except for one surface, which is optically coupled to the photomultiplier tube via a lucite light pipe and a silicon seal. The interior surfaces of the aluminium can are coated with a diffusely

reflecting material such as titanium dioxide, so that all of the light from a scintillation pulse reaches the photomultiplier tube. The height of the output pulse of the photomultiplier is proportional to the energy deposited in the crystal by the incident gamma ray. One or more electronic gates, known as spectrometers, can be used to sort the pulses and pass on only those due to total absorption of gamma rays emitted by the isotope it is desired to detect. These pulses can be counted by a scaler, or a ratemeter can be used to display the average number of pulses received in a given time interval. A single channel pulse height analyzer was used to determine the energy spectrum counted in this study, and a ratemeter was used to count the pulses during profile scanning.

An unshielded scintillation counter crystal will detect background radiation, which comprises that derived from outer space (cosmic radiation) and that from the natural radioactive contamination of the surroundings. The background radiation may make the detection of low levels of radioactivity in a subject difficult. This problem can be eliminated by placing the counting apparatus in a standard low background steel room, but this entails the use of a shield weighing some 20 to 50 tons. In clinical work, however, the shielding requirements are not generally so great, as doses of up to a microcurie of gamma-emitting radioisotope can be used. Hence partial lead and steel shields have been designed

such that direct background radiation cannot reach the counter crystal. Such so-called "shadow shielding" was used in the machines in this study.

VITAMIN B12 ABSORPTION MEASURED BY WHOLE BODY COUNTING

The principles of whole body counting were first applied to the study of vitamin B12 absorption by Reizenstein et al. (1961). Since that time, and apart from the reports by the present author and his colleagues, studies have been made on this subject by Bozian et al. (1963), Callender et al. (1966), Tappin et al. (1966), Meyer et al. (1968), Naversten et al. (1969), Boddy et al. (1969), Irvine et al. (1970), and Cottrall et al. (1971). Heyssel et al. (1966) have used whole body counting to study the assimilation of vitamin B12 from natural foodstuffs and to estimate the minimal daily need for the vitamin in man. Reizenstein et al. (1961) and Boddy et al. (1969) have reported on double isotope methods whereby the absorption of free and intrinsic factor bound vitamin B12 can be studied simultaneously.

There are considerable advantages to studying vitamin B12 absorption by whole body counting. In the first place, it is possible to measure absorption quantitatively; this can only be done otherwise by the tedious faecal excretion method or perhaps by a double isotope hepatic uptake method. In addition, there is no need to collect urine, stool, or blood samples, the presence of

renal disease is irrelevant, and no "flushing" doses of nonradioactive vitamin B12 are given, thereby avoiding metabolic and haematologic changes resulting from that procedure. Finally, the radioactivity left in the body can be measured directly and with a high degree of sensitivity, so that very small doses of radioactive material, in the range of 0.1-0.2 μ c, can be used. As noted by Glass (1963), however, with modern isotopes this last point is of little importance.

The main errors inherent in the whole body counting method have been discussed by Reizenstein et al. (1961), who considered that there were three. First, there was the statistical error inherent in the measurement of radioisotopes, which was 3.0% of the final count for 1.0 μ c of ^{58}Co and 3.2% of the final count for 1.0 μ c of ^{60}Co under the conditions used by these authors. This error is generally very small and, as estimated by Naversten et al. (1969), amounted to about 2.0% which included the errors of patient positioning and counting statistics. A second and greater error, however, was that due to the redistribution of the isotope within the body over the first few hours after giving the test dose and before any excretion of radioactivity had occurred. This results in difficulty in deciding on the 100% value. Reizenstein et al. (1961) noted a mean error of minus 4.1% of the initial count over 1-8 hours after administration of the oral test dose to 6 subjects. (range minus 11.1% to plus 4.9%). Warner and Oliver (1966) gave

a 100% value error of $\pm 3.0\%$ for ^{58}Co ; Tappin et al. (1966) a maximum value of $\pm 10.0\%$ for ^{58}Co , though this was minimized if the 100% value was taken at 3 hours; Naversten et al. (1969) a value of $\pm 13.0\%$ for ^{57}Co ; and Boddy et al. (1969) errors of $\pm 2.7\%$ for ^{57}Co and $\pm 3.4\%$ for ^{58}Co . All these errors were derived from a variable number of repeated readings within the first 24 hours after giving the test dose, usually within the first 6 hours. The third error was that of unabsorbed but as yet unexcreted radioactivity at the time of the final count, which was usually seven days after the test dose was given (range 5-14 days). Vitamin B12 excretion in the faeces has not generally been appreciable more than seven days after ingestion (Halsted et al. 1956) and in the study of Reizenstein et al. (1961) amounted to only 1.1% of the initial count from the seventh to the twenty-second day, which was not considered very significant. Naversten et al. (1969) assessed the importance of this factor in 17 subjects by carrying out counts on the seventh and fourteenth days; they found the decrease in retention of ^{57}Co vitamin B12 during this second week to be 2.0% ($\pm 3.0\%$) or 4.0% ($\pm 5.0\%$), depending on the energy range used for counting, and concluded that only a very small amount of radioactive vitamin B12 remains unexcreted after the seventh day.

Although the errors described above are real ones which must be taken into account in considering results, they are on the whole not very serious, especially in clinical situations. Furthermore, as

Reizenstein et al. (1961) point out, these areas of error can be defined by measurement.

Until fairly recently, it was generally considered that ^{58}Co was the only isotope suitable for use in whole body counting studies. This opinion derived from the fact that, as noted previously, the use of either ^{56}Co or ^{60}Co resulted in relatively large body doses of radiation, while ^{57}Co was considered unsuitable for whole body counting from a technical point of view on account of the comparatively low energy of its gamma rays. More recently, Boddy et al. (1968, 1969) and Naversten et al. (1969) have reported the successful use of ^{57}Co in whole body counting studies, including studies of absorption (Boddy et al. 1969, Naversten et al. 1969). This successful use of ^{57}Co will be supported by the results described below.

The results which have been obtained by various authors are shown in Tables III and IV. As can be seen (Table III), though no studies on the reproducibility of absorption tests in individual subjects have been done, the range of vitamin B₁₂ absorption is very wide, varying from 21% to 98% of the test dose. The separation between control subjects and those with pernicious anaemia is on the whole very good, though occasional overlapping results occur, and Irvine et al. (1970) showed that a good separation between achlorhydric subjects with and without pernicious anaemia could be

TABLE III: Results of Vitamin B12 absorption tests
by whole body counting

AUTHOR	TEST DOSE OF V-B12	CONTROLS		PERNICIOUS ANAEMIA				ACHLORHYDRIA		FOLIC ACID DEFICIENCY		MALABSORPTION			
		No.	Abs. ¹ (%)	No.	Abs. (%)	No.	Abs. (%+IF ²)	No.	Abs. (%)	No.	Abs. (%)	No.	Abs. (%)	No.	Abs. (%+IF)
Bozian et al. (1963)	0.5 µg	5	70.0 (45-80)	18	3.3 (0-17)	-	-	-	-	-	-	-	-	-	-
Tappin et al. (1966)	1.0 µg	27	46.4 (21-78)	11	9.6 (0-34.8)	7	26.0 (15.5- 45.3)	-	-	-	-	7	16 (3.9- 45.1)	3	19.3 (10.3- 33.6)
Meyer et al. (1968)	1.0 µg	-	-	8	13.1 (1.4- 32.9)	7	31.4 (7.6- 41.5)	-	-	7	28.1 (0.4- 46.7)	-	-	-	-
Naversten et al. (1969)	1.0 µg ³	14	55.0 (34-79)	5	5.2 (4-7)	4	64.3 (62- 69)	-	-	-	-	-	-	-	-
Irvine et al. (1970)	0.5 µg	35	64.0 (24-98)	27	9.9 (0-25)	-	-	35	51.3 (22- 79)	-	-	-	-	-	-
Cottrall et al. (1971)	1.1 µg	17	49.0 (30-81)	14	9.8 (0-22)	10	44.0 (27- 56)	-	-	6	32.0 (13-50)	6	16.0 (1-28)	-	-

¹Absorption.

²Intrinsic factor.

³Approximately. Dose varied slightly between subjects. 57Co Vitamin B12 used.

achieved. Considerable increase in absorption could be demonstrated when intrinsic factor was added to the test dose in subjects with pernicious anaemia, and this was not found in 3 subjects with intestinal malabsorption (Tappin et al. 1966). Table IV shows the results of two studies where a double isotope absorption test was used, one form of isotopic vitamin B12 being free and one being bound to intrinsic factor. In both studies there was a very significant increase in the absorption of the isotopic vitamin B12 bound to intrinsic factor in subjects with pernicious anaemia, the single low value (4.0%) in the study of Reizenstein et al. (1961) being in a patient who had become resistant to intrinsic factor following therapy with it. Furthermore, as shown in the study of Boddy et al. (1969), exchange of the bound and unbound isotopes did not seem to occur. The diminished absorption of the intrinsic factor bound isotopic vitamin B12 in control subjects found by Reizenstein et al. (1961) has been suggested as being due to technical factors (Boddy et al. 1969).

Heyssel et al. (1966) have used whole body counting to study the absorption of food vitamin B12, specifically vitamin B12 derived from lean meat and liver of sheep, and to compare its absorption with that of crystalline vitamin B12 (cyanocobalamin). They found that food vitamin B12 was absorbed as well as crystalline vitamin B12, and that there was a failure of absorption of food vitamin B12 in pernicious anaemia. Furthermore, they suggested that the capacity to absorb the vitamin may diminish with age. When

TABLE IV: Double Isotope Vitamin B12 Absorption Test
by whole body counting

AUTHOR	TEST DOSE of V-B12	CONTROLS			PERNICIOUS ANAEMIA			
		No.	Abs. (%)	No.	Abs. (%+IF) ²	No.	Abs. (%)	No.
Reizenstein et al. ³ (1961)	0.1 µg	10	61 (38-80)	10	40 (27-53)	3	3 (0-8.8)	3
Boddy et al. ⁴ (1969)	0.4 µg ⁵	6	44.5 (27- 71.8)	--	--	7	4.2 (1.7-7.1)	7
	0.25 µg	13	40.5 (21.3- 55)	13	49.4 (24.6- 73.5)	12	7 (1.8- 11.8)	12
							28.9 (9.7- 49.2)	

¹Absorption

²Intrinsic Factor

³58Co Vitamin B12 and 60Co Vitamin B12 used.

⁴57Co Vitamin B12 and 58Co Vitamin B12 used.

⁵Only 58Co Vitamin B12 used.

testing the absorption from liver pâté containing 38 μ g of radioactive natural vitamin B12, they found that 6 young normal subjects absorbed 4.1 μ g (range 1.9-6.3 μ g), that 4 old normal subjects absorbed 1.7 μ g (range 0.9-2.3 μ g), and that 5 subjects with pernicious anaemia absorbed 0.68 μ g (range 0-1.41 μ g).

Comparison of Whole Body Counting with Other Methods of Measuring Vitamin B12 Absorption.

There have been reports in which whole body counting has been compared with other methods of measuring vitamin B12 absorption. Callender et al. (1966) showed that there was a good relation between the results obtained by the whole body counting and faecal excretion methods, provided that instances in which inadequate faecal collections had occurred were detected and excluded. These results will be discussed in relation to those obtained in this study. Tappin et al. (1966) compared the results of whole body counting and urinary excretion tests carried out simultaneously. They found a reasonable correlation between the results obtained. The results of urinary excretion tests gave a continuous spread of values over a wide range from 1% to 33%, whereas whole body counting values indicated two groupings: a group containing the controls, who absorbed more than 35% of the test dose, and an abnormal group who absorbed less than 22%. Cottrall et al. (1971) confirmed these results and showed that there was also a reasonable correlation between the whole body counting and urinary excretion results and the 8 hour plasma radioactivity

level. Cottrall et al. (1971) also commented on the poor reproducibility obtained between successive absorption tests.

SUMMARY

There are two types of whole body counters, and of these the solid crystal type is the more versatile and more widely used. A solid crystal counter was used in the study described below.

Most solid crystal counters utilise a thallium-activated sodium iodide crystal as a detector of gamma radiation. Incident gamma radiation evokes the production of photons of light in the crystal, which are converted proportionally to electrical impulses and amplified by a photomultiplier. Electronic gates, known as spectrometers, are used to screen out all impulses but those in a desired energy range. Impulses may be counted on either scalers or ratemeters.

Detector crystals will detect all gamma radiation, including that from cosmic sources and natural surrounding objects; this background may interfere with counting procedures. In clinical work the interference due to background radiation can be reduced sufficiently by limited lead and steel shields.

Whole body counting has been used by a few groups of investigators to measure vitamin B12 absorption. Overall, subjects with pernicious anaemia can be distinguished from control or achlorhydric subjects with relatively little overlapping of results. Exogenous intrinsic factor will restore vitamin B12 absorption to normal in pernicious anaemia, and double isotope tests have been devised to

demonstrate this.

There are sources of error in measuring vitamin B12 by whole body counting. The most important of these involve the determination of the 100% value and the problem of unabsorbed and unexcreted radioactivity.

OBJECTIVES OF THE STUDY

The objectives of this study were as follows: (1) to develop the whole body counting method of measuring vitamin B12 absorption quantitatively; (2) to investigate the sources of error of the method, particularly errors due to variation between machines, to estimation of the 100% value, and to the stool and urine loss of radioactivity; (3) to try to detect errors due to failure to excrete unabsorbed radioactivity; (4) to define the limits of normal absorption and determine whether previously reported wide ranges are due to variable absorption between subjects or in individual subjects; (5) to define the range of absorption in pernicious anaemia; (6) to determine the effect of food on vitamin B12 absorption; (7) to study the relation of vitamin B12 absorption to intrinsic factor secretory capacity where the latter is impaired.

MATERIALS AND METHODS

THE WHOLE BODY COUNTERS

Three machines were used in this study. The first was built in the Medical Physics Department of the Royal Infirmary, Edinburgh (R.I.E. I), and this machine was later modified to make the second machine (R.I.E. II). The third machine was a mobile whole body counter belonging to the Scottish Research Reactor Center (S.R.R.C.) which was used for machine comparison studies in cooperation with Dr. Keith Boddy of the Scottish Research Reactor Center, East Kilbride, Glasgow.

R.I.E. I.

The Apparatus: The detector used a sodium iodide crystal 12.5cm in diameter and 9cm thick. It incorporated a 12.5cm diameter photomultiplier tube, so that the assembly was an integral crystal-photomultiplier unit. Results have been obtained using a scaler which supplies the external high tension and incorporates a single-channel pulse-height analyser.

The crystal-photomultiplier unit was suspended from a wheeled gantry (Fig. 2) spanning the couch on which the subject lay; it was positioned 45cm above the couch. A 2.5cm thick lead shield weighing 50kg surrounded the crystal (Fig. 2), and this reduced the background by about 50%, being more effective at lower energies. The shield, which was 30cm long, 28cm wide, and 19cm high, had a collimating effect such that the field of view at couch level was just sufficient to include the width of the subject being counted. At a point 20cm on

FIG. 2: Machine R.I.E. I, showing the crystal-photomultiplier unit, surrounded by a lead shadow shield, suspended on a wheeled gantry above the couch used for counting subjects



either side of the mid-line the sensitivity had fallen to 85% of the maximum. The shield had no collimating effect in a longitudinal direction. The uniformity of response in this direction largely depended on the length of the scan; variation over the central 100cm was within $\pm 5\%$. This central area would include a subject's trunk, where the majority of the ^{58}Co activity would be found. Under extreme conditions, with a source placed in positions corresponding to the subject's head or feet, the sensitivity dropped to 75% of maximum. Uniformity could have been increased by increasing the length of the scan, but this would have been offset by the high background associated with the particular geometry used.

Counting Method: The dependence of the count rate on the position of radioactivity in the body was reduced by making the crystal scan over the subject. This was done by moving the detector over the length of the couch at a steady speed of 1cm per second. In addition, total counts were made with the subject both prone and supine to correct for redistribution of radioactivity in an anterior-posterior direction. One hundred and eighty centimeter scans were done in each direction in both the prone and the supine position, the total number of counts being accumulated by the scaler. The total counting time was 12 minutes.

Energy Range for Counting: The optimum energy range for counting was determined by making measurements over a small ^{58}Co source which was placed in various positions inside a water tank 20cm deep; this was to mimic the redistribution of radioactivity inside a human. Measurements were made in various energy ranges (Table V), the 0.40-0.65

TABLE V: *Relative Counting Rates from a small ^{58}Co source showing the effect of attenuation and inverse square effects in different energy ranges*

Channel	I	II	III	II+III	I+II+III
Energy Mev	0.2-0.4	0.4-0.65	0.65-1.00	0.4-1.00	0.2-1.00
Depth 0 cm	75%	100	100	100	93
5	96	99	94	96	100
10	100	96	77	86	92
15	96	99	94	96	100
20	75	100	100	100	93
$s^2/B \times 10^{-3}$	1.6	1.8	2.6	4.2	5.3

$$s^2/B = \text{Signal}^2/\text{Background}$$

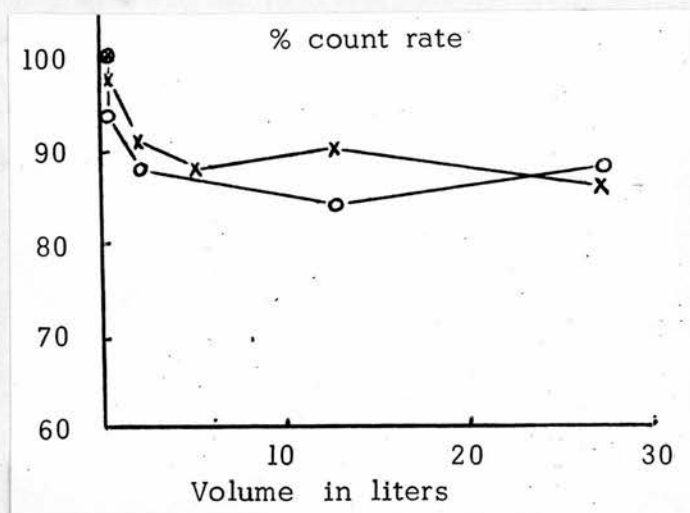
Mev range including the 0.51 Mev annihilation peak, and the 0.65-1.00 Mev range including the 0.81 Mev gamma-ray peak. Table V shows the variation in the relative count rate as the depth of the radioactivity source was increased, the counts from both sides being added together. Table V also shows the $\text{Signal}^2/\text{Background}$ (S^2/B) value. Although the 0.40-0.65 Mev range gave the smallest variation in response, counting over a wide range (0.20-1.00) was only slightly more variable and gave twice the sensitivity. The advantages of working over this wide range would be increased by conditions in which the background is lower, as when a more effective shield is used.

The effect of diluting a small source of ^{58}Co was also studied (Fig. 3). Curve 0-0 shows the effect of placing a 1.0 μc source at the center of increasing volumes of water. The reduction of the count rate due to the dilution effect (curve X-X) was considered to be acceptably small.

In summary, when absorption tests were carried out, final results were calculated for the full 0.20-1.00 Mev range, calculations always being done in the 0.20-0.66 Mev and 0.66-1.00 Mev ranges as a checking method.

Sensitivity: This was not of prime importance, as the counter was designed for clinical application. However, the sensitivity, defined as the radioactivity which will give a count above the background equal to three times the standard deviation of the total background count in 20 minutes, is 0.014 μc --i.e. 1.4% of the dose of 1.0 μc (see below) used in clinical tests.

FIG. 3: *Relative counting rate from a 10ml source containing 1.0 μ c of ^{58}Co*



0—0 Placed at the centre of increasing volumes of water.

X—X Diluted in increasing volumes of water.

R.I.E. II.

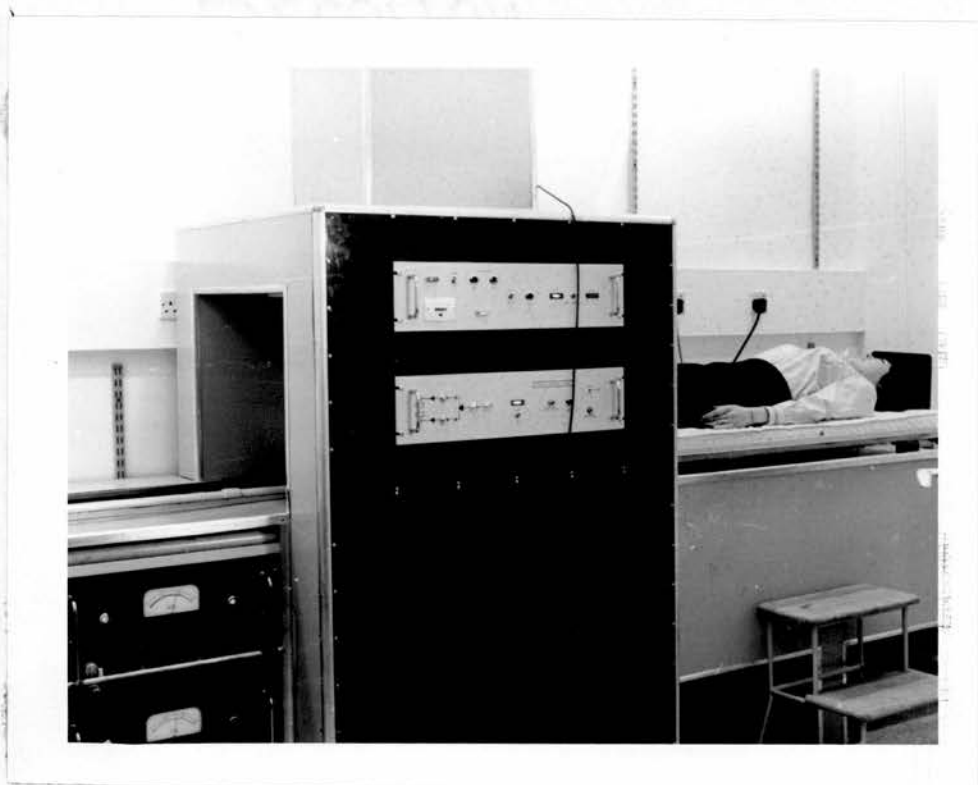
This machine was a modification of R.I.E. I described above. An additional detector crystal, identical to that described above, was added so that the subject could be counted from above and below simultaneously. The subject could therefore be counted in the supine position only. In this machine the detectors were in a fixed position and the subject was moved between them on a motorised couch. In addition, the shielding of the apparatus was increased (Fig. 4), using 10cm of lead, to give a shadow shield effect similar to that described by Warner and Oliver (1966) with the addition of side walls which also contained 10cm of lead. The lead sheets, mounted on steel plates, surrounded the crystals and covered an area 70cm across and 95cm along the couch. This protection was enough to prevent radiation from the surroundings reaching the crystals. Apart from these modifications, the procedures were as described for R.I.E. I above.

This machine (R.I.E. II) was used in the machine comparison study with machine S.R.R.C. described below. In this study ^{57}Co vitamin B12 was also used, and this isotope was counted in the 0.03-0.17 Mev energy range.

S.R.R.C.

This whole body counter is the one described in detail by Boddy (1967). It utilized a single sodium iodide crystal detector, 29.2cm in diameter and 10.2cm thick, surrounded by a shadow shield of 10.2cm of lead. The subject was scanned from head to feet in both the prone and supine positions, passing beneath the detector on a motorised couch.

FIG. 4: *Machine R.I.E. II, showing the greatly increased lead shadow shield which enclosed the two crystal-photomultiplier units*

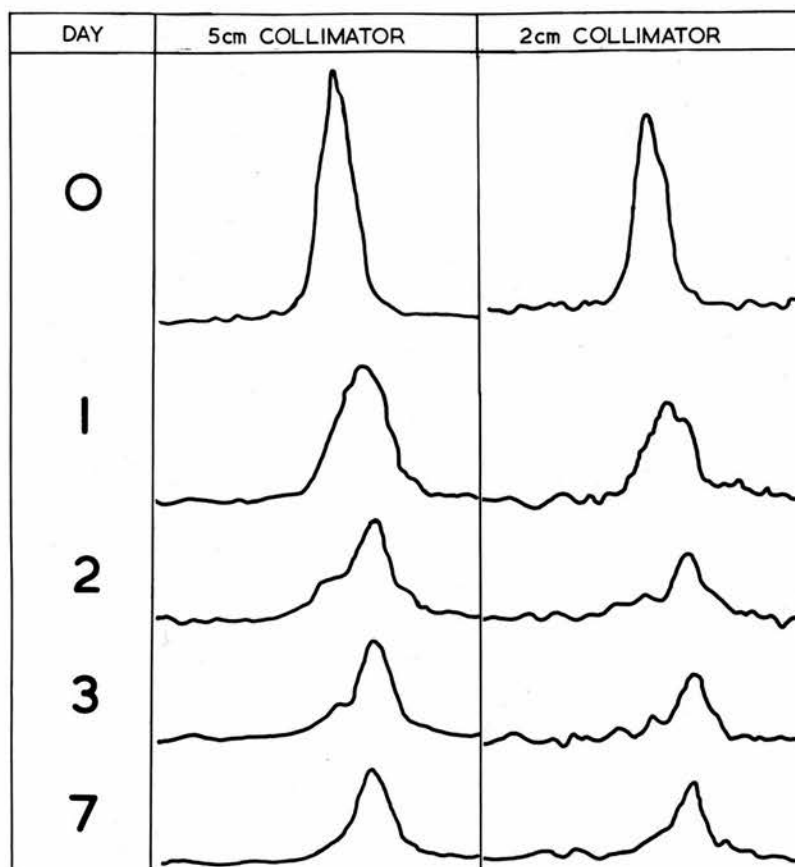


Measurements were made in the 0.20-1.00 and 0.66-1.00 Mev ranges for ^{58}Co and in the 0.03-0.17 Mev range for ^{57}Co .

PROFILE SCANNING

An attempt was made to assess the completeness of excretion of unabsorbed radioactivity from the bowel using sequential profile scanning in conjunction with the whole body counting. Two machines, R.I.E. I and R.I.E. II, were used in this study, although the majority of tests were done with R.I.E. II. Both machines gave similar results. Scans were carried out at a speed of 1cm per second, with the crystal(s) collimated by a parallel-sided lead collimator; the collimator had a lip at the edges giving an effective thickness there of 10cm. Initially, scans were carried out with collimator slit widths of 2cm and 5cm, but it was found that the tracing obtained with the 2cm setting was irregular. Although the 2cm setting gives better resolution, the irregularity of the tracing probably resulted from the small amount of radioactivity used, resulting in a relatively high statistical fluctuation in the count rate. Thus, after the initial tests, all scans were done with 5cm collimator settings. Fig. 5 compares the scans obtained in a subject who had normal vitamin B12 absorption using both 2cm and 5cm collimator settings. For profile scanning the crystal(s) were brought closer to the subject. In the single crystal counter the crystal to couch separation was reduced from 45cm to 38cm, while in the double crystal apparatus the distance between the crystals was reduced from 84cm to 70cm. In the single crystal counter, separate scans were done

FIG. 5: *The effect of collimator settings on the regularity of the profile scan*



Note the relative irregularity with the 2cm setting, resulting in difficulty in detecting deflections due to body radioactivity.

with the patient supine and prone, while in the double crystal counter the combined output from the detectors was used to give a single scan. The output from the crystal(s) was counted on a rate-meter, set to 300 counts per second full scale deflection and a time constant of one second, and printed out on a paper chart recorder.

Absorption tests were done as described below, using ^{58}Co as the radioactive tracer, with the exception that profile scans and whole body counts were done on as many days as possible up to the seventh day. During the whole of this period 24 hour collections of urine and stool were made. In some subjects, at the completion of the test, a proportion of the test dose was put into the rectosigmoid region through a soft rubber catheter; profile scans were then carried out to try to detect this.

ABSORPTION TEST PROCEDURES

Tests Performed Without Food: Each subject injected 1.0 μC of ^{58}Co contained in 0.5 μg of cyanocobalamin with 100ml of water after a 12 hour fast. Subjects were recounted on the seventh day and this recorded the retained radioactivity, which was mainly in the liver.

Tests Performed with Food: After a 12 hour fast, the subject ate a vitamin B12-free meal interspersed with sips of the ^{58}Co vitamin B12 in 100ml of water. The meal consisted of rice, carrots, turnip, corn oil, and spices, followed by peaches and 50ml of orange juice containing 10g of sugar. Only one patient disliked the meal, which was usually eaten within 15 minutes. *Euglena gracilis* assay showed that the whole meal contained less than 0.02 μg of vitamin B12.

It should be noted that, to minimize urinary excretion of some of the absorbed radioactivity, no injections of vitamin B12 were administered during any test. Senocot (1.2g) was always given on the third day, and subjects were questioned regarding bowel movement before the final count.

Use of the Counter with Subjects: Background and standard measurements were made over 10 minute periods on each occasion that a whole body count was done. The standard was 1.0 μ c of 58Co in 5 liters of water in a polythene container. Before a test dose was administered, the background was measured with the subject in position by accumulating the counts obtained during two scans in the supine position and two in the prone position for machine R.I.E. I, and two scans in a supine position for machine R.I.E. II. This allowed correction to be made for any reduction in background due to the presence of the subject and also for any radioactivity already present in the subject. After administration of the test dose, counting was started immediately to determine the count rate due to the activity, which should be almost entirely in the stomach. In 29 subjects the variation in count rate during the first 6 hours was tested by repeated counting within that period. In 16 subjects a 58Co label was used for the vitamin B12, and in 13 subjects a 57Co label. Measurements were repeated after 7 days, and the new ratio of patient to standard count rate was used to estimate the amount of radioactivity retained at that time. To obtain the subject background, the instrument background was corrected using data obtained on the first day.



Reproducibility: This was tested by carrying out two or more tests of vitamin B12 absorption in successive weeks. Studies of reproducibility were done where test doses were given both with and without food.

MEASUREMENT OF URINARY AND FAECAL RADIOACTIVITY

Measurements of the urinary excretion of radioactivity after the ingestion of the test dose were made in 14 control subjects. Twenty-four hour collections of urine were made, these being continued for two days in 3 of the subjects, and for seven days in 11 of the subjects. Measurements of the faecal excretion of radioactivity were made in 48 control subjects and in 8 subjects with pernicious anaemia. All stool passed after the ingestion of the test dose was collected for seven days; in 45 instances the collections were divided into separate 24 hour periods.

Stool radioactivity was measured in a ring of 12 Geiger-Muller tubes (Type GM 26 Pb) shielded by 4cm of lead shot. Urine activity was measured by counting 20ml aliquots in a well-type sodium iodide crystal counter (Tracerlab Gamma-guard). The crystal was 7.5cm in diameter and 7.5cm thick with a hole 2.5cm in diameter and 5.0cm deep.

GASTRIC FUNCTION TEST

Augmented histamine secretion tests were carried out after the method of Kay (1953) on all but the control subjects. In each case the position of the nasogastric tube was checked radiologically, and every attempt was made to ensure a full collection of gastric juice by paying particular attention to the subject's position, by the use of

deep breathing, and by frequent aspiration of the nasogastric tube by hand.

Gastric Juice Collection: After aspiration of the fasting secretions, a basal 60 minute collection of juice was made. One of two gastric stimulants was then given by the subcutaneous route: histamine acid phosphate 0.04mg/kg of body weight or pentagastrin 6.00µg/kg of body weight. When histamine was used, mepyramine maleate (100mg) was given intramuscularly 20-30 minutes beforehand to minimize side-effects. Gastric juice collections were made over 10 minute periods for one hour after gastric stimulation. Acid and intrinsic factor production was measured over this one hour period. During the hour after stimulation, samples were collected onto ice so that intrinsic factor as well as acid secretion could be measured. Mucus was removed from all samples by centrifugation at 2,000rpm for 20 minutes, the pH was raised at once to 10 with 0.1N sodium hydroxide for 30 minutes to destroy pepsin (see below) and then returned to pH 7.0 with 0.1N hydrochloric acid before storage at -20°C. Acid content was measured during titration with 0.1N sodium hydroxide to pH 7.0 using a Beckman model 72 pH meter. Subjects whose gastric juice never fell below pH 6.0 were designated as achlorhydric.

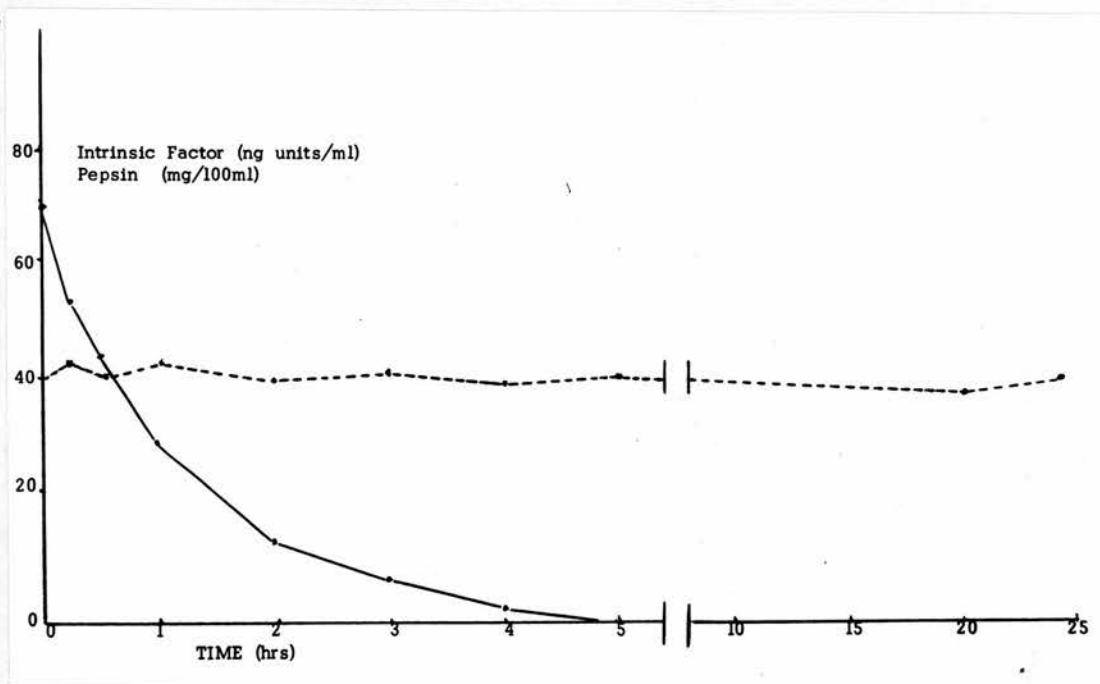
Intrinsic Factor Stability: The extent to which all the procedures for collecting gastric juice are necessary is not well known, and experiments were therefore carried out to investigate how critical they are to obtaining reproducible results. In the destruction of intrinsic factor in acidic gastric juice, three main factors have been considered

important--temperature, pH, and peptic activity--and these are the factors which were investigated. All intrinsic factor and pepsin measurements were made on gastric juice collected after histamine or pentagastrin stimulation.

In the first experiment the intrinsic factor and pepsin concentrations in samples of acidic gastric juice collected from a subject with a duodenal ulcer and kept at 37°C, room temperature, and 4°C were investigated over 24 hours. The pH of the gastric juice was 2.1, which is optimal for pepsin activity. Aliquots from each sample were removed at once and at intervals for up to 24 hours for estimation of intrinsic factor and pepsin activity. The results are shown in Figs. 6-8. Each value plotted represents the mean of two assays showing a variation of less than 5%. At 37°C the intrinsic factor concentration was reduced by half in one hour and had disappeared completely within five hours (Fig. 6). At room temperature (Fig. 7) and at 4°C (Fig. 8) intrinsic factor concentration was unchanged at 24 hours. At all these temperatures pepsin was stable.

In the second experiment the effect of raising the pH on inhibiting pepsin activity, or on destroying it, was investigated. Acidic gastric juice samples were collected at room temperature in 9 subjects. Pepsin was measured at pH 2.0 in these samples, and the measurements were then repeated after the juice had been stored at pH 7.0, or at pH 7.0 after an initial period of 30 minutes at pH 10.0. Each value represents the mean of two assays showing a variation of less

FIG. 6: *Survival of intrinsic factor and pepsin
in native gastric juice (pH 2.1) at 37°C*



----- = Pepsin

———— = Intrinsic Factor

FIG. 7: Survival of intrinsic factor and pepsin in native gastric juice (pH 2.1) at room temperature

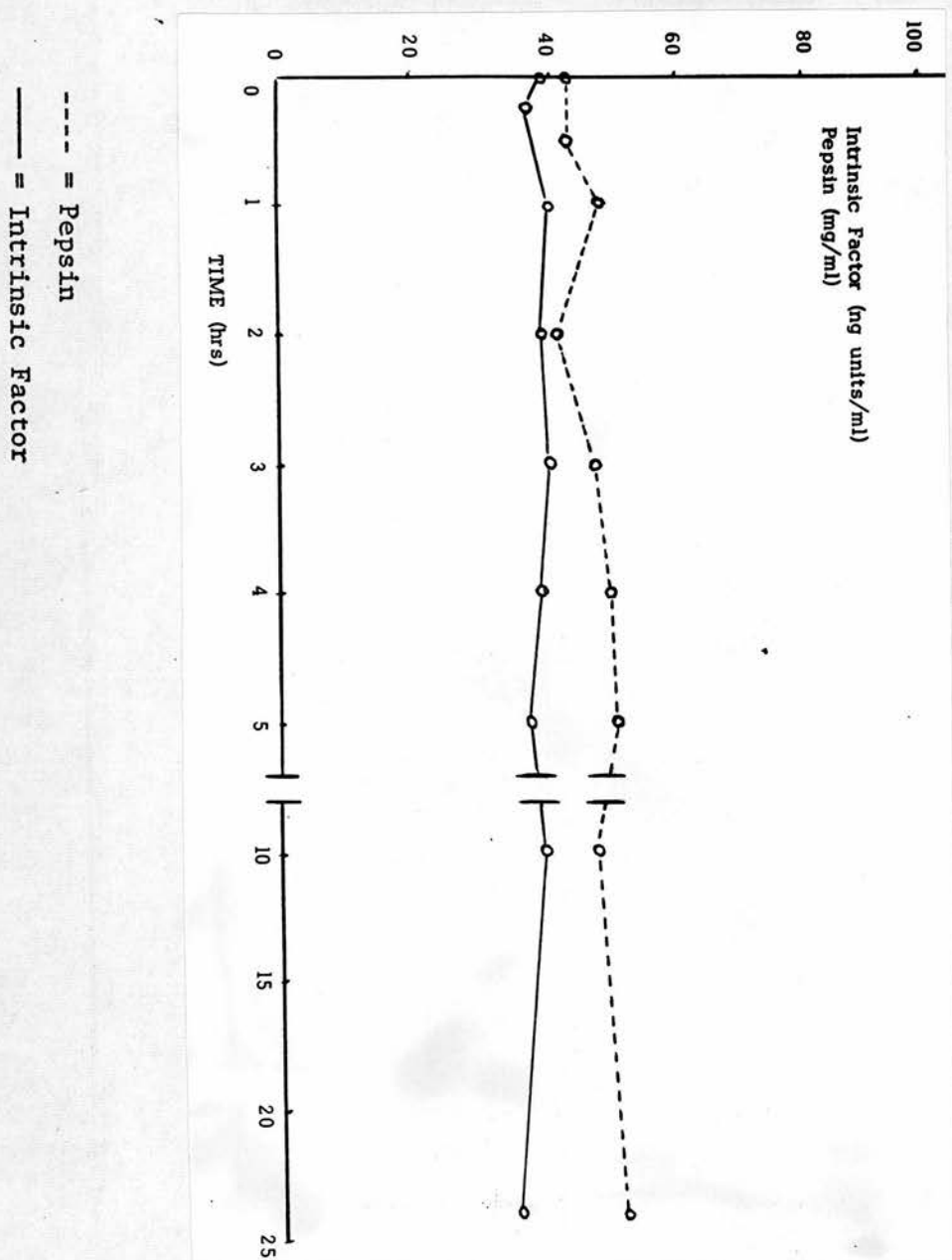
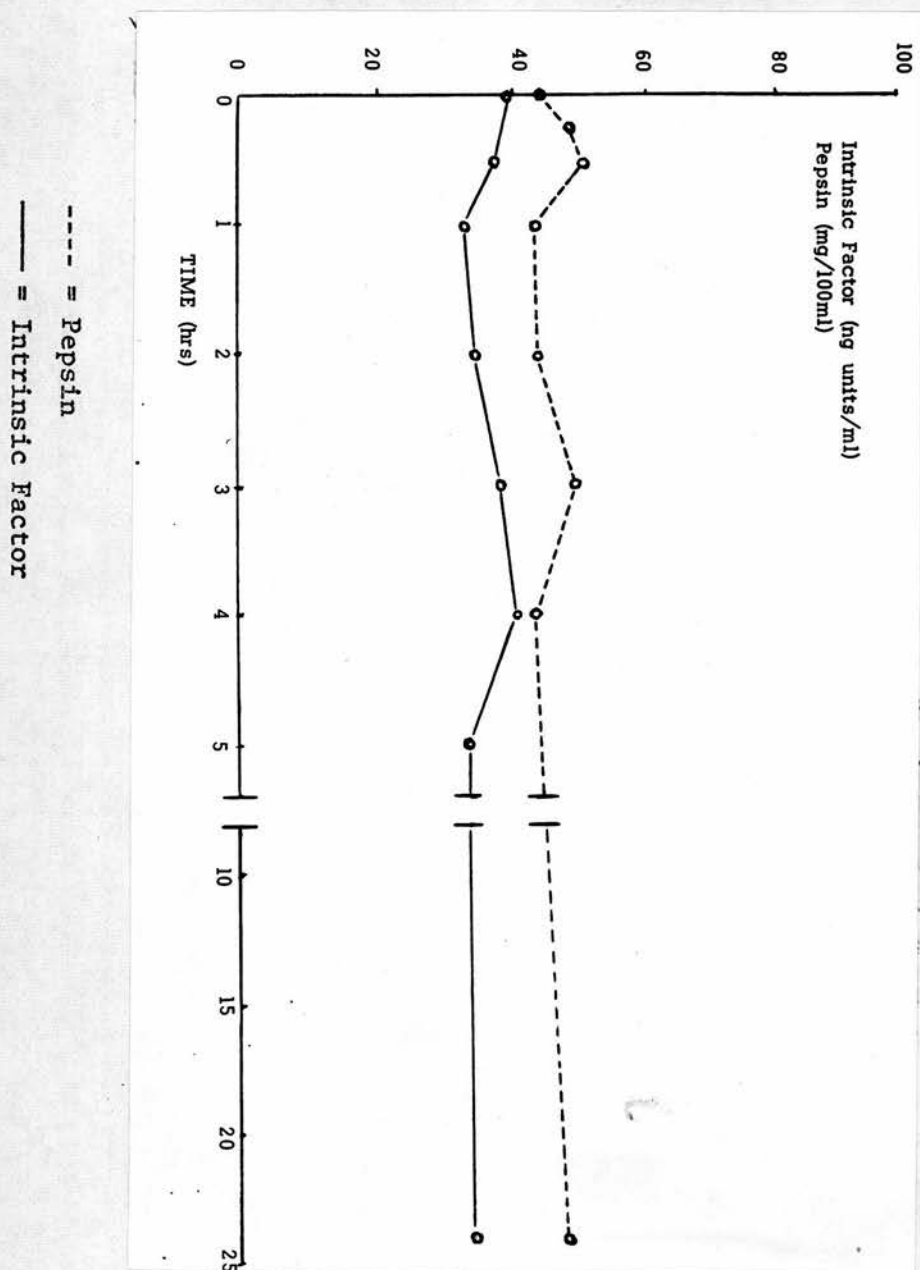


FIG. 2: Survival of intrinsic factor and pepsin in native gastric juice (pH 2.1) at 4°C



than 10%. Fig. 9 shows that pepsin is not totally destroyed at pH 7.0 but is after 30 minutes at pH 10.0. The effect of raising the pH of gastric juice to 10.0 on intrinsic factor concentration was tested in the same way, and the results in Fig. 10 show that there is no loss of intrinsic factor activity for up to two hours at this pH.

In the third experiment the effect of a low pH on intrinsic factor at room temperature was tested. Acidic gastric juice was collected as described above, and pepsin was destroyed by raising the pH to 10.0 for 30 minutes. Intrinsic factor was measured in the original sample, after which the pH was reduced to 2.0 and intrinsic factor measured at intervals for up to 24 hours. Fig. 11 shows that reducing the pH to 2.0 did not alter intrinsic factor concentration over this period.

As it is standard practice to store gastric juice samples at -20°C before intrinsic factor assay, the effect of this on intrinsic factor activity was investigated. Pepsin was destroyed in gastric juice samples as described above, after which they were frozen at -20°C and thawed twice. This did not alter intrinsic factor activity.

From these experiments it was concluded that the method of collecting gastric juice used gave an optimal preservation of intrinsic factor.

Gastric Stimulants: During the course of this study it became apparent that pentagastrin was the gastric stimulant of choice for these tests, as it produced far fewer side-effects than histamine even

FIG. 9: Effect on pepsin levels of raising the pH of gastric juice to pH 7.0 and pH 10.0

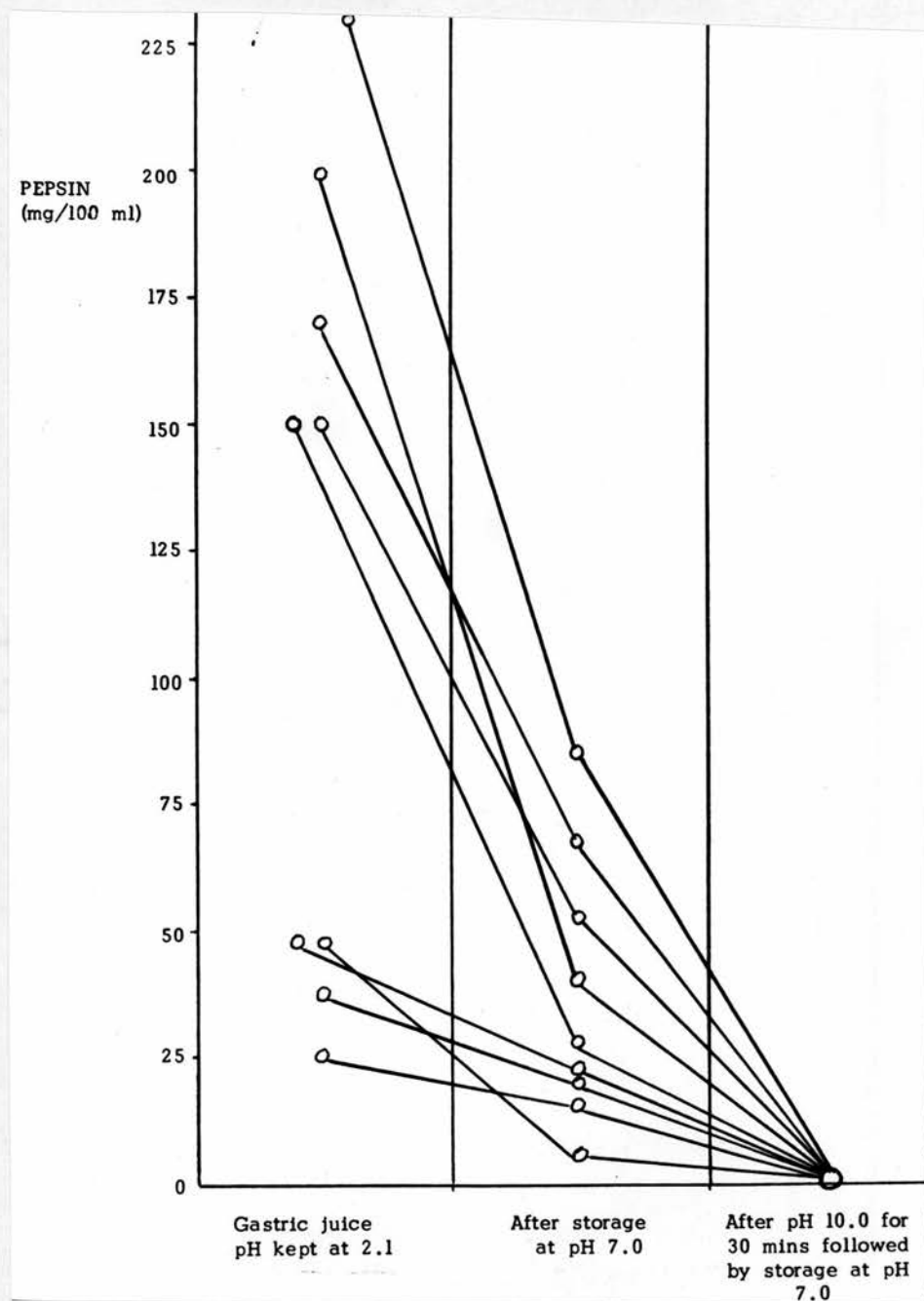


FIG. 10: *Effect on intrinsic factor survival
of raising pH to 10.0*

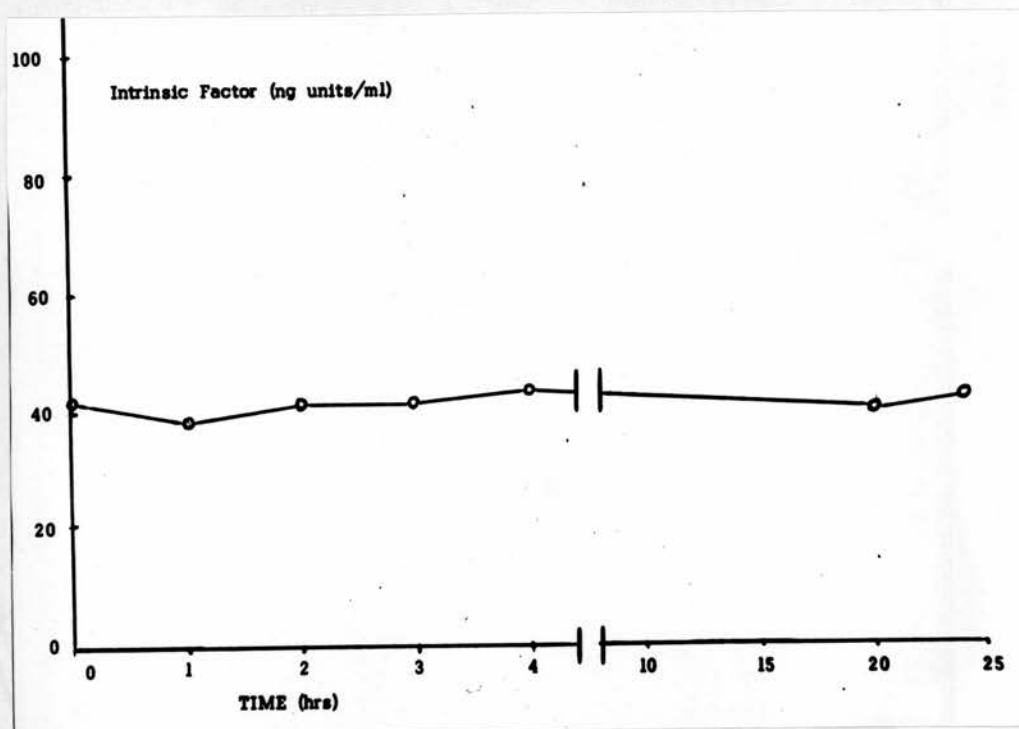
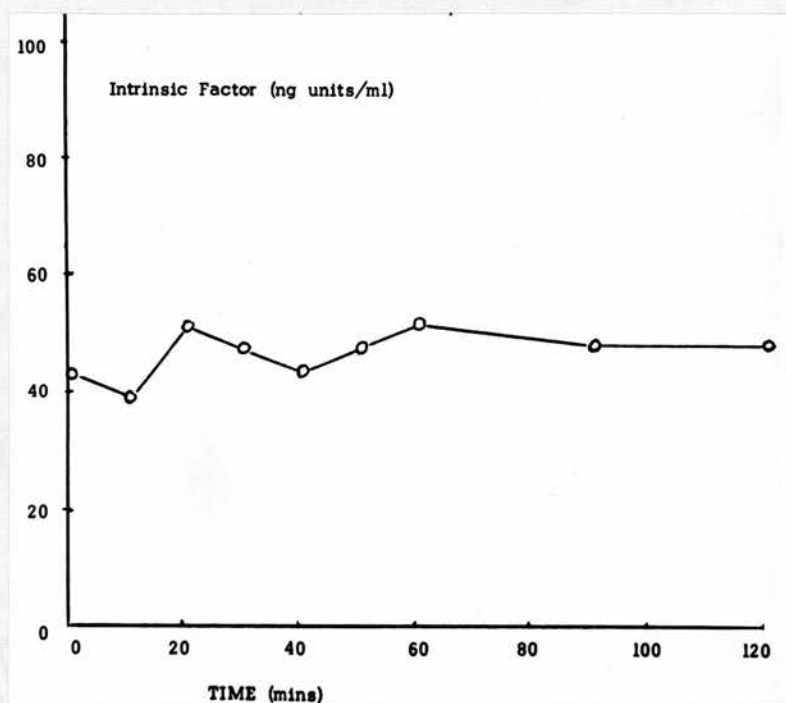


FIG. 11: *Effect on intrinsic factor survival of reducing pH to 2.1 after the destruction of pepsin*



when mepyramine had been given beforehand. It had been shown by a number of different workers (Makhlouf et al. 1966, Wormsley et al. 1966, Multicenter Pilot Study 1967, Konturek 1967) that the two stimulants, histamine and pentagastrin, gave equivalent results for acid secretion used in the doses stated above. However, this had not been shown for intrinsic factor secretion, and an investigation was therefore carried out in 15 subjects chosen so as to cover a wide range of gastric acid and intrinsic factor secretion. Each of these subjects had had a gastric function test carried out as described above, using histamine as the stimulant, for diagnostic purposes. In 13 subjects the histamine (0.04mg/kg of body weight) had been given subcutaneously and in 2 by continuous intravenous infusion (0.04mg/kg of body weight/hour) after the method of Lawrie et al. (1964). Each subject was then asked to volunteer for a further test. This was performed within three days of the first test, and pentagastrin, 6.0µg/kg of body weight subcutaneously or 6.0µg/kg of body weight/hour intravenously, was given. Single subcutaneous injections of pentagastrin were adjusted to approximately 3ml (Wormsley et al. 1966). Acid and intrinsic factor were measured as described.

The subjects studied included 4 with pernicious anaemia, 3 with duodenal ulcer, 2 with achlorhydria (one of whom had idiopathic iron-deficiency anaemia), 2 with pyloroplasty and vagotomy, in one of whom the vagotomy was shown by an insulin test (Ross and Kay 1964) to

be incomplete, and one each with gastric ulcer, gastric carcinoma, pancreatic carcinoma, and iron deficiency anaemia secondary to blood loss.

Table VI shows the acid and intrinsic factor outputs in the post-histamine and post-pentagastrin hours in the 13 subjects where the stimulant had been given subcutaneously. The range of acid secretion covered was 0-49mEq. There was a good correlation between the acid, intrinsic factor, and gastric juice volumes produced in response to the two stimulants; the relevant correlation coefficients and probability values are shown in Table VII. The only gross difference was between the acid productions in subject 11 (Table VI); in this case marked contamination of the gastric juice with bile was seen in the test where pentagastrin was used.

Figs. 12 and 13 show the paired infusion tests carried out on two subjects, one with duodenal ulcer (subject 14) and one with pyloroplasty and incomplete vagotomy (subject 15). In both these subjects, "steady state" acid and intrinsic factor outputs in the second hour of stimulation were similar. During the first hour of stimulation both intrinsic factor and acid secretion rose sooner after pentagastrin than it did after histamine. In subject 15 the peak response to intrinsic factor was lower after pentagastrin, but this subject had already secreted over 8000ng units in the 40 minutes before pentagastrin was given.

Only one subject (subject 4) complained of nausea after re-

TABLE VI: The output of acid and intrinsic factor in one hour in response to histamine (0.04 mg per kg) and pentagastrin (0.6 µg per kg) given on separate occasions

Subject Number	Diagnosis	Acid output (mEq.) in response to		Intrinsic factor output after					
		Histamine	Pentagastrin	Histamine			Pentagastrin		
				Vol.	Conc. (ng per ml)	Total ng units	Vol.	Conc. (ng per ml)	Total ng units
1	Pernicious anaemia	0.0	0.0	69.5	1.4	97	32.7	0.7	23
2	Pernicious anaemia	0.0	0.0	6.2	0.0	0	22.0	6.5	143
3	Pernicious anaemia	0.0	0.0	63.5	0.0	0	69.5	0.0	0
4	Pernicious anaemia	0.0	0.0	57.0	3.7	211	67.0	0.0	0
5	Idiopathic iron-deficiency anaemia	0.0	0.0	26.0	14.0	364	44.5	14.0	623
6	Idiopathic achlorhydria	0.0	0.0	54.0	39.0	2106	48.5	27.0	1310
7	Gastric carcinoma	0.0	0.0	87.0	38.0	3306	97.0	32.5	3153
8	Pancreatic carcinoma	6.2	11.2	109.0	103.0	11,227	165.0	73.0	12,045
9	Iron-deficiency anaemia	10.6	11.0	121.0	71.7	8675	238.0	33.0	7854
10	Pyloroplasty and vagotomy	16.7	19.1	273.0	11.0	3003	223.0	19.5	4349
11	Gastric ulcer	20.4	4.6	287.0	27.0	7749	236.0	32.0	7552
12	Duodenal ulcer	39.0	38.5	317.0	76.0	24,092	302.0	65.0	19,630
13	Duodenal ulcer	49.0	47.8	456.0	72.0	32,832	426.0	86.0	36,636

TABLE VII: *The correlations between gastric juice volumes, acid secretions, and intrinsic factor secretions in one hour after stimulation with histamine and pentagastrin on separate occasions in thirteen subjects, as well as the correlations between acid secretion and intrinsic factor secretion in all tests done.*

Measurement correlated	r^1	p^2
Gastric juice volume	0.94	< 0.001
Acid secretion	0.96	< 0.001
Intrinsic factor secretion	0.99	< 0.001
Acid and intrinsic factor secretion	0.93	< 0.001

¹ r = correlation coefficient

² p = probability of a chance association between the observations made

FIG. 12: Effect of histamine and pentagastrin on intrinsic factor (a) and acid (b) secretion in a subject with duodenal ulceration

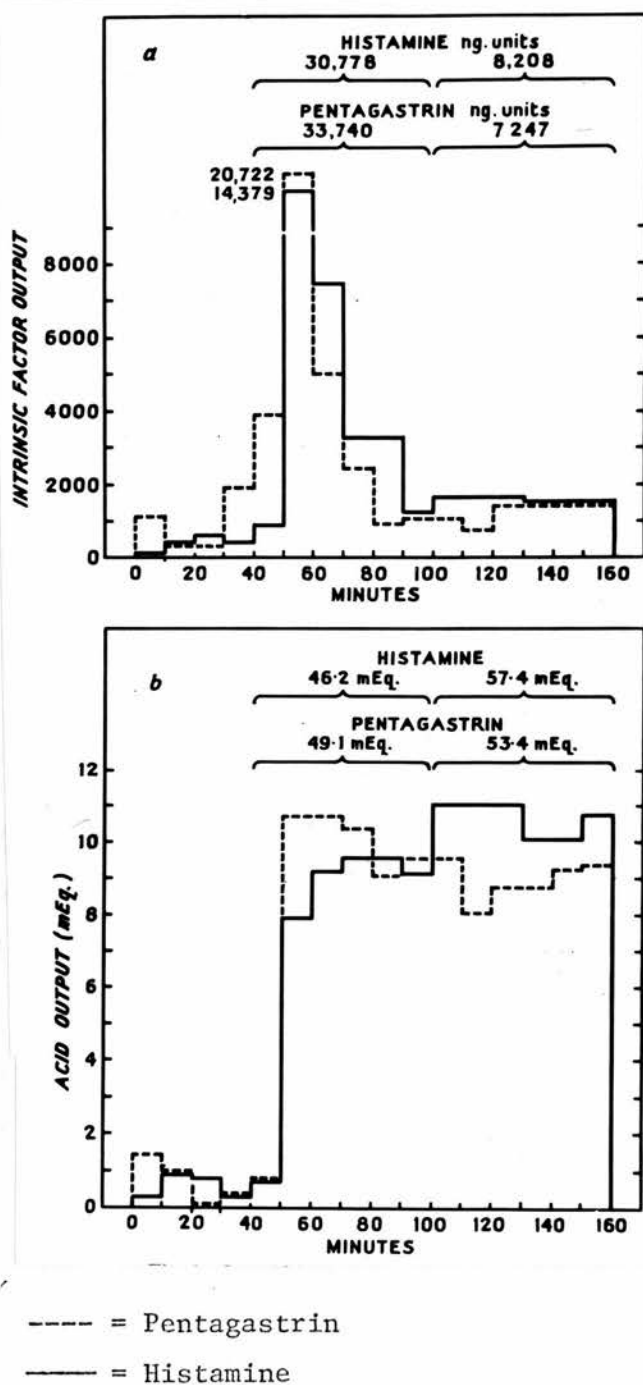
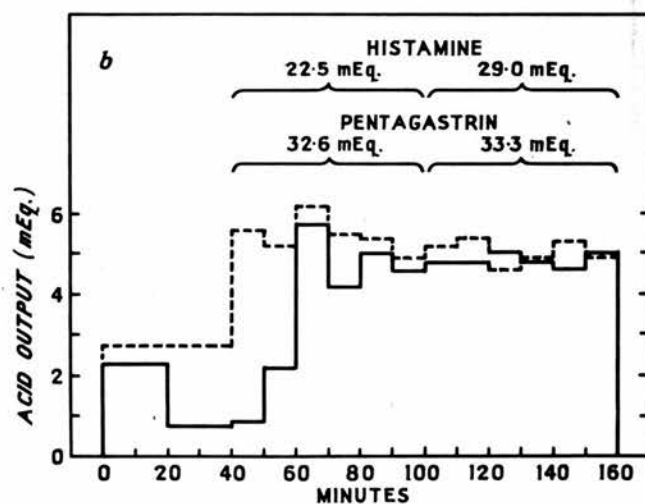
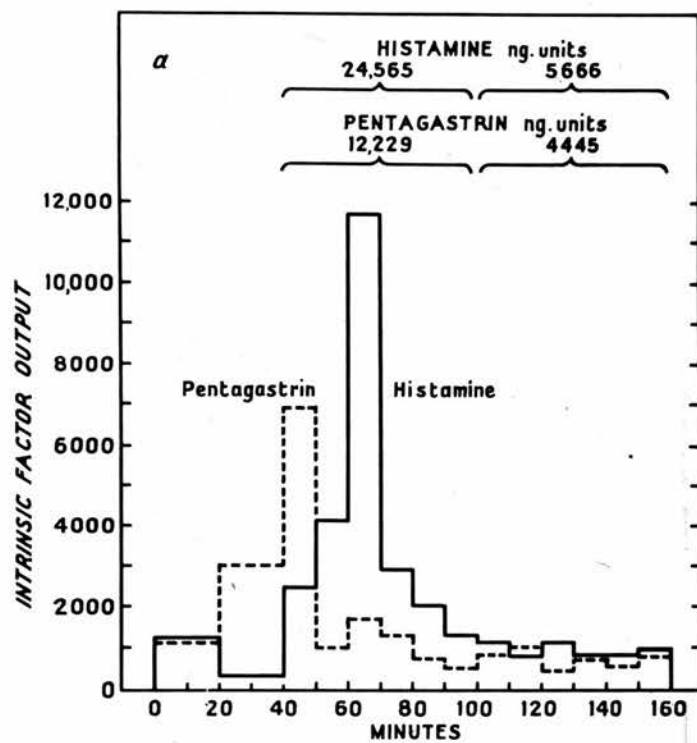


FIG. 13: *Effect of histamine and pentagastrin on intrinsic factor (a) and acid (b) secretion in a subject who had had a pyloroplasty and incomplete vagotomy*



----- = Pentagastrin

———— = Histamine

ceiving pentagastrin, in contrast to eight subjects who had striking flushing and/or headache after receiving histamine.

In this part of the study acid responses to pentagastrin were similar to those obtained after histamine, which accords with the work of others (Makhlouf et al. 1966, Wormsley et al. 1966, Multicenter Pilot Study 1967, Konturek 1967). The intrinsic factor outputs were also comparable. As the side-effects of histamine may be severe despite antihistamine cover, it was concluded that pentagastrin was the stimulant of choice for these tests.

INTRINSIC FACTOR MEASUREMENT

The method used for intrinsic factor measurement was that of Ardeman and Chanarin (1963), which is based on the observation that the ability of intrinsic factor in gastric juice to bind radioactive vitamin B12 is abolished by type I antibody to intrinsic factor. This antibody is found in the serum of many patients with pernicious anaemia. Gastric juice was collected for intrinsic factor estimation as described above, and all assays were carried out in duplicate at pH 7.0.

The initial mixture of reagents in the assay used in this study is summarised in Table VIII. Antisera to intrinsic factor capable of blocking the uptake of more than 75ng of vitamin B12 per ml were used, so that an excess of type I intrinsic factor antibody would be present. This ensured that the sole variable in the system was the amount of intrinsic factor in the gastric juice. These reagents (Table VIII) were allowed to mix for 10 minutes, after which 1 ml of ^{57}Co (1.0 $\mu\text{C}/\mu\text{g}$) vitamin B12 (400ng/ml) was added to each tube. After a further 20 minutes, 0.5ml of 20% weight per volume of acid washed and

TABLE VIII: *Reagent combination for assay of intrinsic factor in gastric juice*

REAGENTS	TEST SAMPLE		SERUM CONTROLS		STANDARD
	1	2	3	4	5
Phosphate buffer (ml)	1.0	1.0	1.5	1.5	2.5
Test gastric juice (ml)	0.5	0.5	-	-	-
Normal serum (ml)	1.0	-	-	1.0	-
Antiserum (ml)	-	1.0	1.0	-	-

Note that all assays are done in duplicate.

reactivated charcoal was added to the test samples and serum controls to remove free vitamin B12. Two minutes later the mixtures were centrifuged for 10 minutes at 2000rpm and the radioactivity in the clear supernatants and the standard measured.

The difference in count rates between the supernatants from mixtures 1 and 4 (Table VIII) gives a measure of the combined intrinsic factor and non-intrinsic factor vitamin B12 binders in the gastric juice under test. The difference in the count rates between the supernatants from mixtures 2 and 3 gives a measure of the non-intrinsic factor vitamin B12 binders in that gastric juice. Hence the difference between these two measurements indicates the amount of intrinsic factor which was in the gastric juice--i.e., where S indicates the count rates from the supernatants of original mixtures (Table VIII), $(S_1 - S_4) - (S_2 - S_3)$ is a measure of the intrinsic factor in the test gastric juice.

The actual amount of intrinsic factor is calculated from the count rate of the standard. All assays were done in duplicate. Only those with a difference of less than 10% were accepted.

PEPSIN MEASUREMENT

The measurement of pepsin activity in gastric juice was made by the method of Hunt (1948) as modified by Bitsch (1966), so that results could be expressed in mg of pepsin.

STATISTICAL ANALYSES

Statistical tests used were as detailed by Bailey (1968) and by Siegel (1956).

SUBJECTS

The subjects used in this study were classified as follows. Patients admitted to hospital for diseases other than those of the

alimentary tract, and who had no evidence from the clinical history or physical examination of bowel disease likely to interfere with small intestinal absorption, were used as control subjects. All of these subjects had normal haemoglobin, haematocrit and white blood cell counts. Achlorhydric subjects were diagnosed on the results of gastric function tests as described above; those with megaloblastic anaemia and malabsorption of vitamin B12 which could be reversed by giving exogenous intrinsic factor, as demonstrated by the urinary excretion test (Schilling ~~et al~~ 1953), were defined as having pernicious anaemia. Gastric ulcer was diagnosed radiologically, Crohn's disease by radiological methods and rectal biopsy, and coeliac syndrome by the demonstration of atrophy of the upper small intestinal villi for no known reason. Patients who had had gastric operations were classified in accordance with the type of surgery which had been done.

Table IX shows the subjects used in the development of the whole body counting method for vitamin B12 absorption and in the clinical studies; the subjects tested with each of the machines used in this stage of the study (see below) are shown separately; Table X shows the total number of procedures carried out on these subjects. Thirty-four subjects were used in the study to compare the results obtained using two machines simultaneously. In these subjects the diagnosis was not important, but subjects with intact stomachs were chosen in such a way that a range of absorption from very low, represented by subjects with pernicious anaemia, to normal would be covered.

TABLE IX: Diagnoses in subjects used in the development of whole body counting
(excluding machine comparison study)

Machine	Tests/ Subject	Test Conditions	ACHLORHYDRIA		GU ²	P ³	INTESTINAL DISEASES					Controls	Total
			PA ¹	No PA			P+V ⁴	PG ⁵	CS ⁶	CD ⁷			
R.I.E. ⁸ I	1	Fasting	5	0	0	8	9	1	0	0	9	32	
R.I.E. ⁸ II	1	Fasting	11	0	1	0	0	5	3	2	19	41	
R.I.E. ⁹ II	2	Fasting	31	12	0	0	0	13	19	0	24	100	
	2	Food	11	6	0	0	0	7	2	0	11	37	
R.I.E. I & II Profile Scanning	1	Fasting	6	0	0	0	0	0	2	1	15	24	
TOTAL SUBJECTS			64	18	1	8	9	26	26	3	78	233	

¹PA = Pernicious anaemia ²GU = Gastric ulcer ³P = Pyloroplasty ⁴V = Vagotomy

⁵PG = Partial gastrectomy ⁶CS = Coeliac syndrome ⁷CD = Crohn's disease

⁸These subjects were used primarily for faecal and urinary vitamin B12 loss studies and for profile scanning.

⁹Subjects had either two fasting tests or two tests with food.

TABLE X: *Total number of procedures in the development of the whole body counting method*

MACHINE	100% VALUE STUDIES	RADIOACTIVITY EXCRETION		REPRODUCIBILITY STUDIES		PROFILE SCANNING	MACHINE COMPARISON STUDIES
		Stool	Urine	Fasting Tests	Food Tests		
R.I.E. I	6	32	0	0	0	0	0
R.I.E. II	23	21	14	100	37	24	0
R.I.E. II and S.R.R.C.	29	0	0	0	0	0	25

RESULTS

THE MEASUREMENT OF VITAMIN B12 ABSORPTION BY WHOLE BODY COUNTING

In this part of the work, particular attention was paid to those aspects of the whole body counting method for measuring vitamin B12 absorption where inaccuracies could occur. This included the estimation of the 100% value, the way in which radioactivity was lost from the body in urine and stool, the detection of unabsorbed radioactivity in the bowel which had not been excreted, the normal range of absorption, the reproducibility of absorption measurements, a comparison of the results of measuring absorption with two different whole body counting machines simultaneously, and a comparison of the results of absorption measurements obtained by the whole body counting and faecal excretion methods done simultaneously.

The 100% Value.

The variation in the 100% value was measured in 29 subjects, 16 of whom were given ^{58}Co vitamin B12 and 13 of whom were given ^{57}Co vitamin B12. Four or five whole body counts were done on each subject in the seven hours following the administration of the test dose. No difference was found between machines R.I.E. I and R.I.E. II, nor was there any difference related to whether or not previous gastric surgery had been done; all the results are therefore presented together.

Table XI shows the results obtained using ^{58}Co vitamin B12. There was no significant difference between the results obtained with the three different counting channels, all giving coefficients of

TABLE XI: Mean Count Rates, Standard Deviations, and Variances
for the 100% value using a 58Co label

Subject	Counting Ranges (Mev)									
	0.20-0.66			0.66-1.00			0.20-1.00			V %
	Mean 100% Count Rate	SD ¹ Counts	V ² %	Mean 100% Count Rate	SD Counts	V %	Mean 100% Count Rate	SD Counts	V %	
1	3631	53	1.8	1239	8	0.9	4839	50	1.0	1.0
2	2501	46	2.2	906	13	1.9	3407	48	1.8	1.8
3	3785	24	0.7	1236	8	0.7	5021	29	0.6	0.6
4	3897	79	2.0	1198	47	3.8	5095	123	3.0	3.0
5	4250	58	1.8	1399	25	2.2	5648	103	1.8	1.8
6	2864	186	6.5	839	64	7.6	3703	245	6.6	6.6
7	2849	249	8.7	851	24	2.8	3701	270	7.3	7.3
8	2508	42	1.8	786	31	3.9	3293	50	1.5	1.5
9	2994	164	5.5	1019	41	1.0	4013	195	4.9	4.9
10	3078	60	2.0	1054	27	2.6	4131	84	2.0	2.0
11	3059	91	3.0	1001	28	1.0	4060	110	1.0	1.0
12	2807	62	1.0	902	38	1.0	3707	98	1.0	1.0
13	2841	435	15.3	921	111	12.1	3887	389	10.0	10.0
14	3306	88	2.7	1144	31	2.7	4451	112	2.5	2.5
15	3448	111	3.2	1161	35	3.0	4609	145	3.2	3.2
16	4033	103	2.6	1367	33	2.4	5400	131	2.4	2.4

¹Standard deviation ²Variance

Mean Variance: 0.20-0.66 Mev = 3.8%
0.66-1.00 Mev = 3.1%
0.20-1.00 Mev = 3.2%

variation below 4%. The largest individual coefficient of variation was 15.3%, and the smallest one 0.7%.

Table XII shows the results obtained using ^{57}Co vitamin B12. The mean coefficient of variation was 6.9%, the largest coefficient of variation being 12.1%, and the smallest one 0.7%.

Loss of Radioactivity from the Body.

The Faeces: Loss of radioactivity in the faeces during the seven days of the test was measured in 45 subjects where faecal collections had been made in separate 24 hour periods. Fig. 14 shows that the mean daily faecal excretion of radioactivity had fallen to 0.3% of the test dose by the seventh day; one subject excreted 5.3% of the test dose on the seventh day, but no other subjects excreted more than 1.5%. Fig. 15 shows that in 69% of patients the maximal daily excretion of radioactivity had occurred by the end of the third day, and that it had occurred in 93% by the end of the fifth day.

Profile scanning: An advantage of measuring vitamin B12 absorption by whole body counting is that in addition to giving quantitative results, collection of stool and urine are not needed. Although the studies described above indicate that, at least where a laxative is given, faecal excretion of unabsorbed radioactivity is largely if not wholly complete in a seven day period, failure of excretion of unabsorbed radioactivity remains a serious potential source of error in the investigation. Tests were therefore done to see whether the technique of profile scanning could be used to detect re-

TABLE XII: *Mean Count Rates, Standard Deviations, and Variances for the 100% value using a ^{57}Co label*

Subject	Mean 100% Count Rate	Standard Deviation Counts	Variance %
1	3963	124	3.2
2	3910	247	6.3
3	3211	388	12.1
4	3952	291	7.2
5	4050	133	4.0
6	4400	281	7.9
7	5876	455	7.7
8	4475	607	13.6
9	3740	118	3.2
10	5917	206	3.5
11	4444	642	14.5
12	6080	301	5.0
13	5184	200	1.0

Counting range 0.03-0.17 Mev

Mean variance = 6.9%

FIG. 14: *Mean daily faecal excretion of radioactivity
(% total dose)*

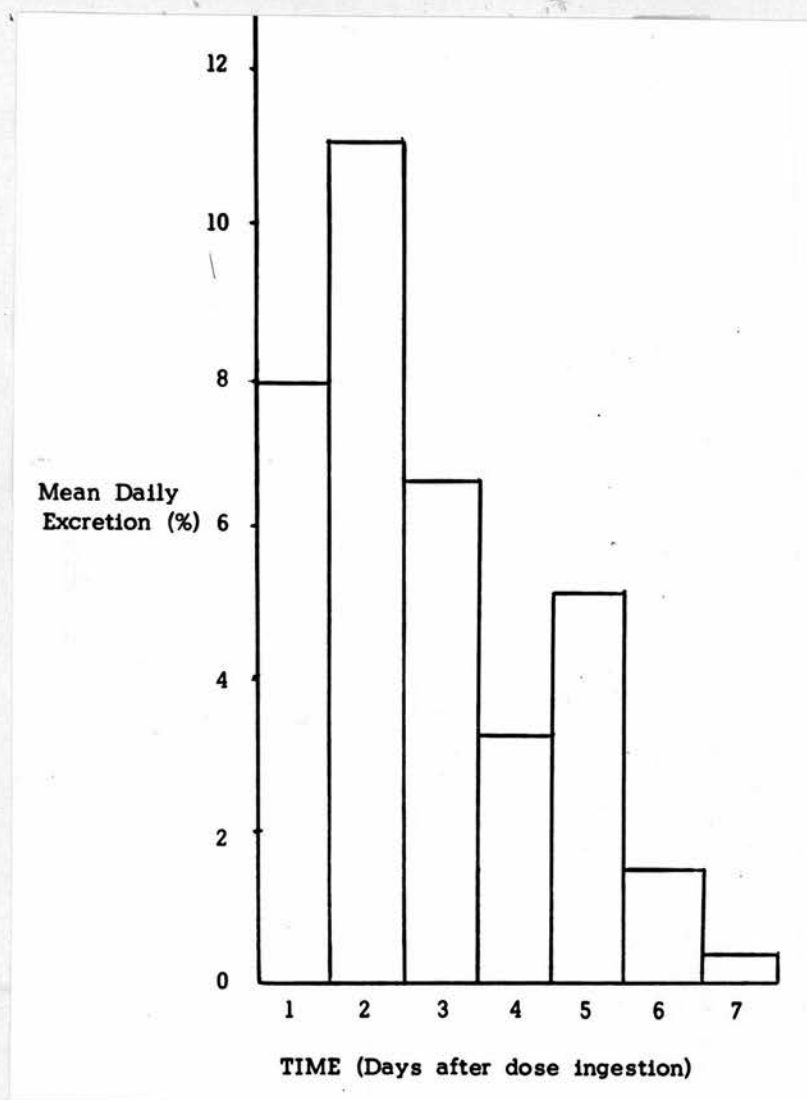
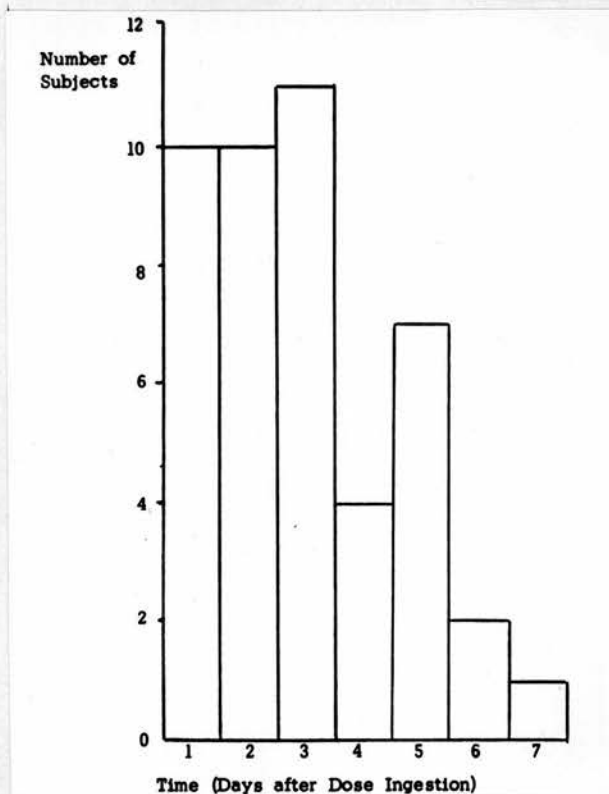


FIG. 15: Days of maximal excretion of radioactivity after the ingestion of the test dose of ^{58}Co vitamin B12



tained unabsorbed radioactivity. The reason for thinking that profile scanning might be a useful adjunct to this whole body counting method was based on the fact that absorbed radioactivity would be found mainly in the liver where it might be sufficiently separated from unabsorbed radioactivity in the bowel to allow the latter to be detected independently.

Twenty-four subjects were studied. Fifteen absorbed more than 40% of the test dose. The remaining nine patients all absorbed 22% or less and included subjects with pernicious anaemia (6), coeliac disease (2), and one patient with Crohn's disease. Representative profile scan patterns from these patients will be illustrated.

Two profile scan patterns were found. The most common occurred in 21 of the subjects and is illustrated in Fig. 16, which shows the head-to-toe scans starting from the right. After the test dose was swallowed, a single peak was obtained over the stomach. Within 24-48 hours this had disappeared to be replaced by two peaks, one over the liver which was called the "liver" peak, and the second, presumed to be in the bowel, which was called an "intestinal" peak. By the sixth day the intestinal peak had disappeared; this disappearance coincided with the biggest fall in whole body radioactivity and the greatest excretion of faecal radioactivity, as shown in Fig. 16.

Three subjects had scans in which only 1 peak was seen during the week. Two of these had severe vitamin B12 malabsorption and the scans of one are shown in Fig. 17. By the sixth day the peak had

FIG. 16: Profile scanning during a vitamin B12 absorption test in a normal subject showing the development of a "double peak" pattern

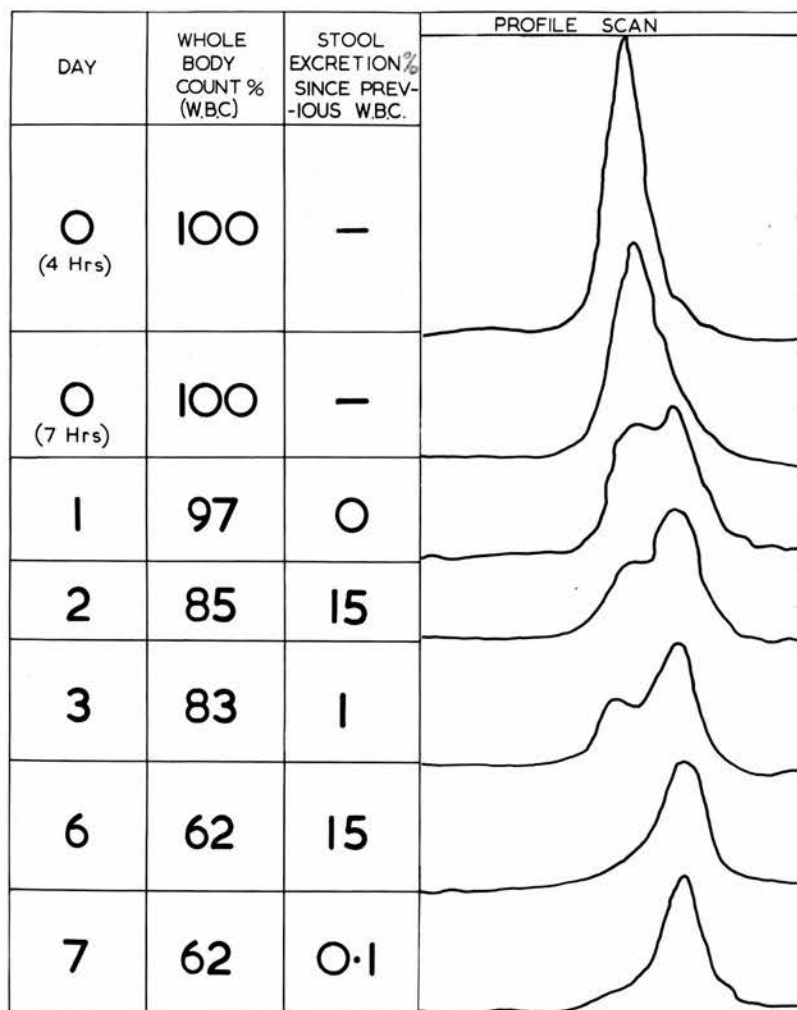
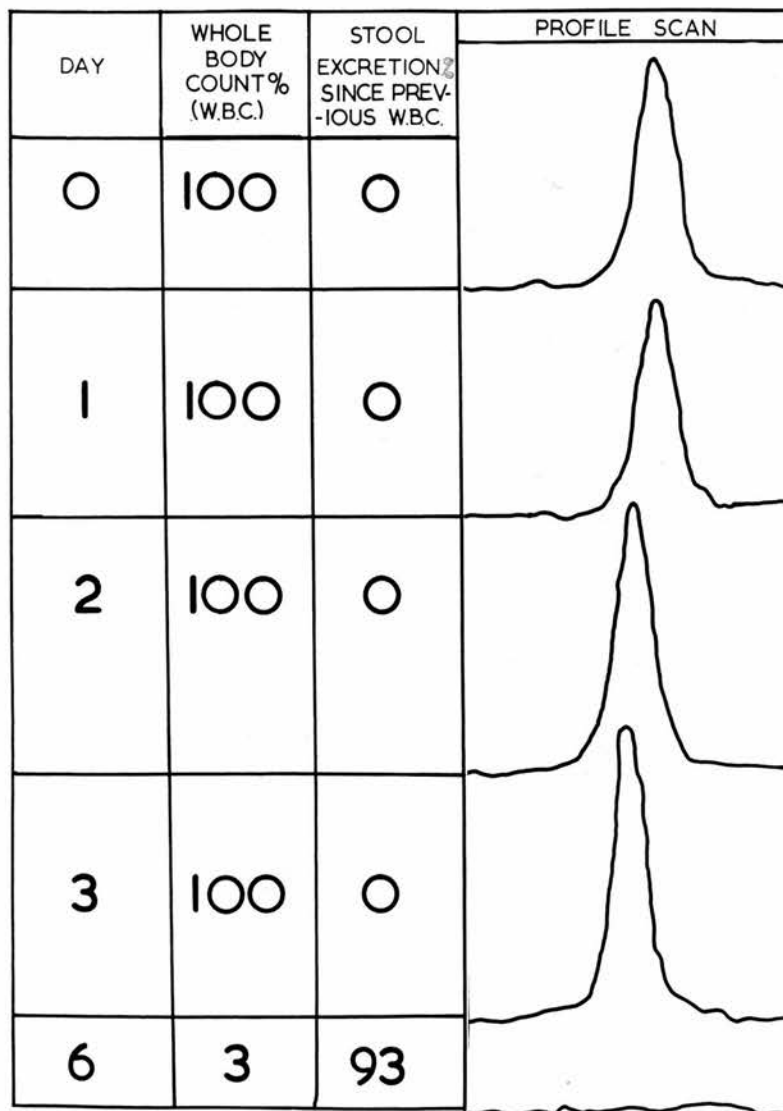


FIG. 17: Profile scanning during a vitamin B12 absorption test in a subject with malabsorption showing a "single peak" pattern due to failure to develop a "liver peak"

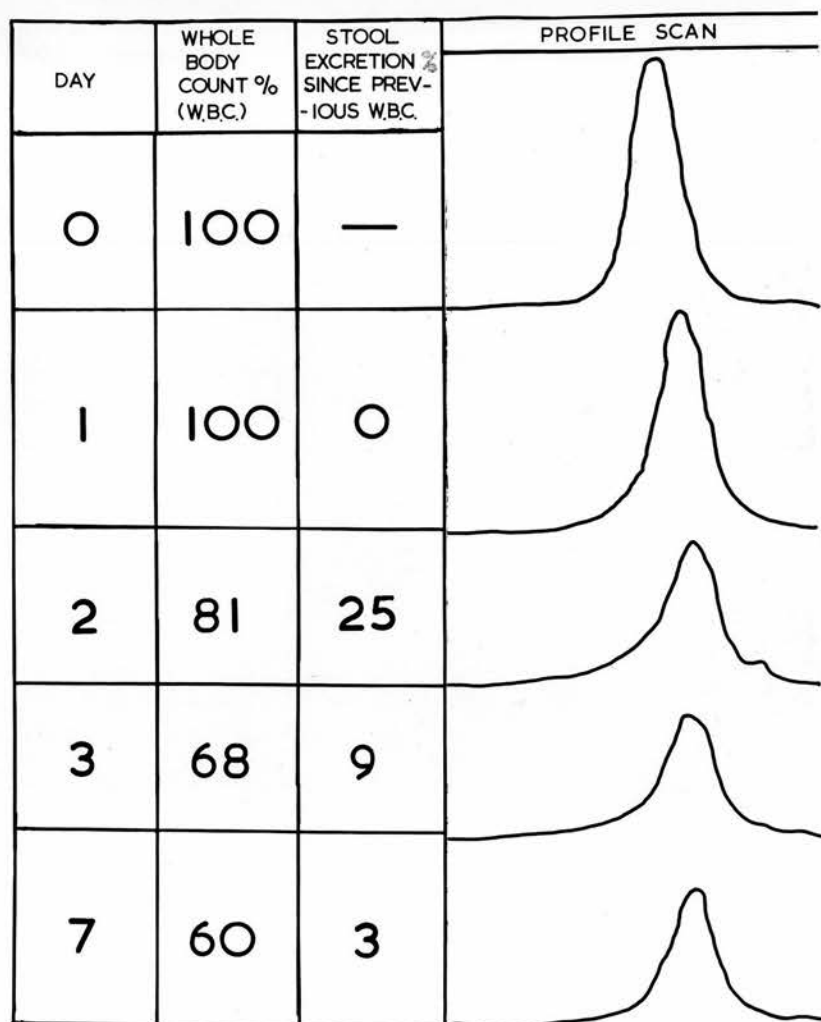


disappeared, after which whole body retention was 3%. This peak therefore represented an "intestinal" peak occurring alone. Fig. 18 shows the only subject in whom normal absorption occurred with a single peak pattern.

Attempts were made to reproduce the "intestinal" peak by introducing small proportions of the test dose into the rectum after the 7th day of scanning. Fig. 19 shows the smallest amount (10% of the test dose) needed to reproduce the peak in the presence of normal absorption. In malabsorption, where the "liver" peak was low, smaller amounts could reproduce an "intestinal" peak; this is shown in Fig. 20 (5% of the test dose).

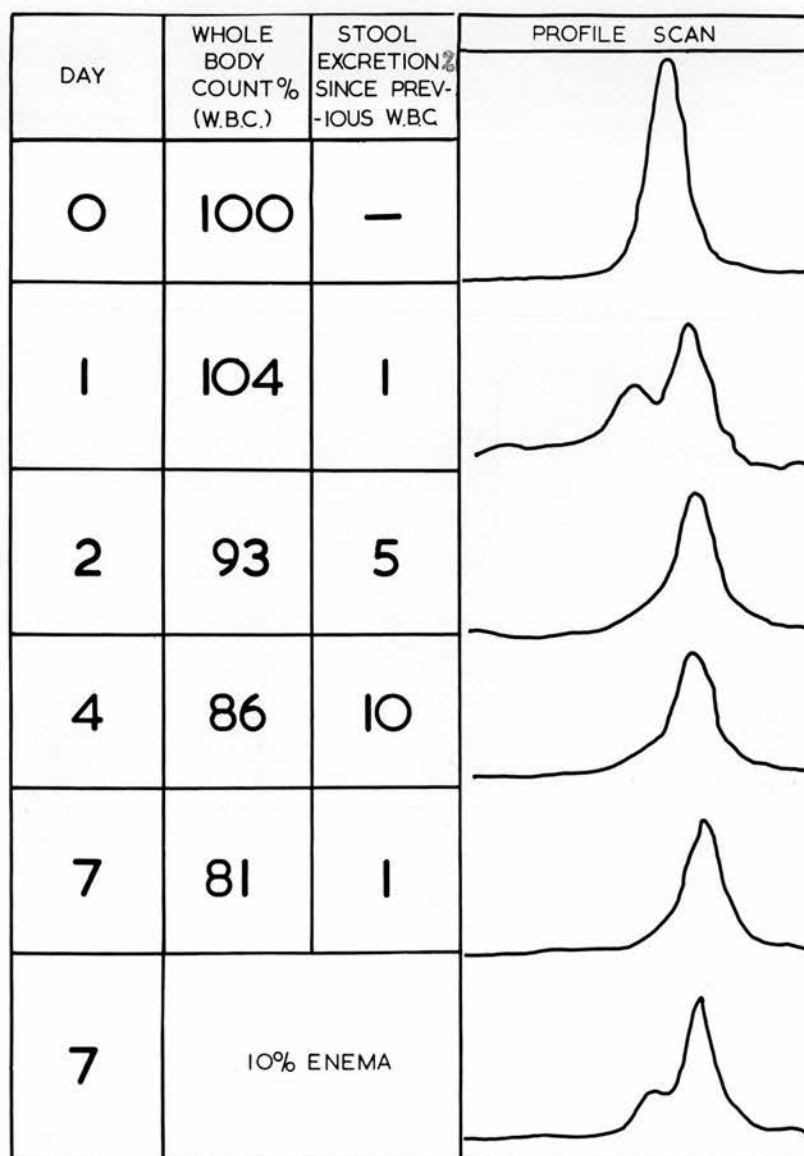
Urine: The possibility that absorbed radioactivity might be lost by urinary excretion during the seven days allowed to elapse while faecal excretion of unabsorbed radioactivity was occurring was investigated in 14 control subjects. Twenty-four hour urine collections were made for 2 days in 3 subjects, all of whom excreted less than 0.25% of the test dose in this period. In a further 11 subjects, twenty-four hour urine collections were made for seven days. Seven subjects excreted less than 1% of the test dose in this period, three subjects excreted from 1% to 3% of the test dose, and one subject excreted 7.2% of the test dose in the seven day period. This last subject had a duodenal ulcer, but was otherwise in good health. He had received no therapy during the test period, and in particular had had no parenterally administered vitamin B12.

FIG. 18: Profile scanning during a vitamin B12 absorption test in a normal subject



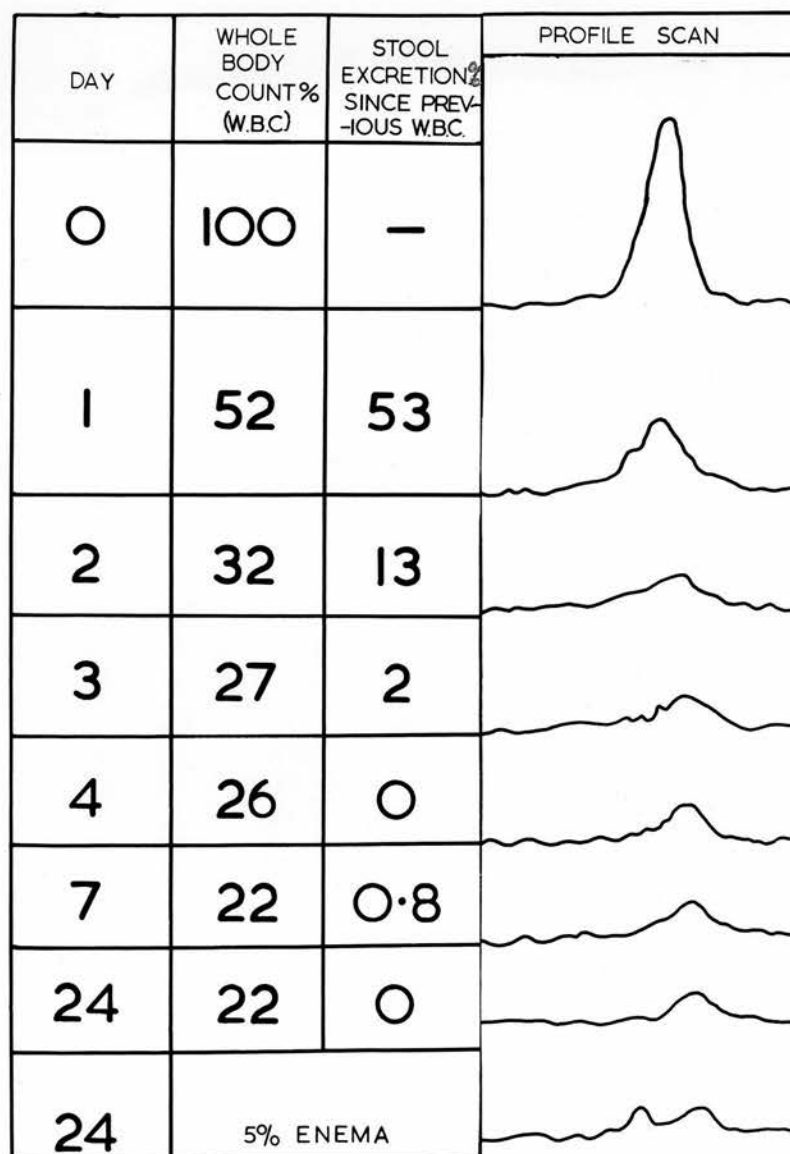
This was the only occasion on which a "single peak" pattern occurred with normal absorption due to failure to visualise an "intestinal peak."

FIG. 19: *Profile scanning during a vitamin B12 absorption test in a normal subject*



The "intestinal peak" could be reproduced with a minimum of 10% of the test dose of ^{58}Co (0.1 μc) placed in the rectosigmoid region.

FIG. 20: Profile scanning during a vitamin B12 absorption test in a subject with malabsorption



The "intestinal peak" could be reproduced with a minimum of 5% of the test dose of ^{58}Co ($0.05\mu\text{c}$) placed in the rectosigmoid region. Note the very small "liver peak."

Normal Absorption and Malabsorption.

In defining the ranges of normal absorption and malabsorption, a comparison was made between the results obtained in control subjects and those with pernicious anaemia. The comparison with control subjects was limited to those with pernicious anaemia because this was the only group of subjects where vitamin B12 absorption would be abnormal by definition; other individual subjects who either had a malabsorption syndrome or had had gastric surgery might or might not malabsorb vitamin B12. No patient with a total gastrectomy was studied. Subjects studied in the fasting state are principally considered in this section, as tests of vitamin B12 absorption are in general done under these conditions.

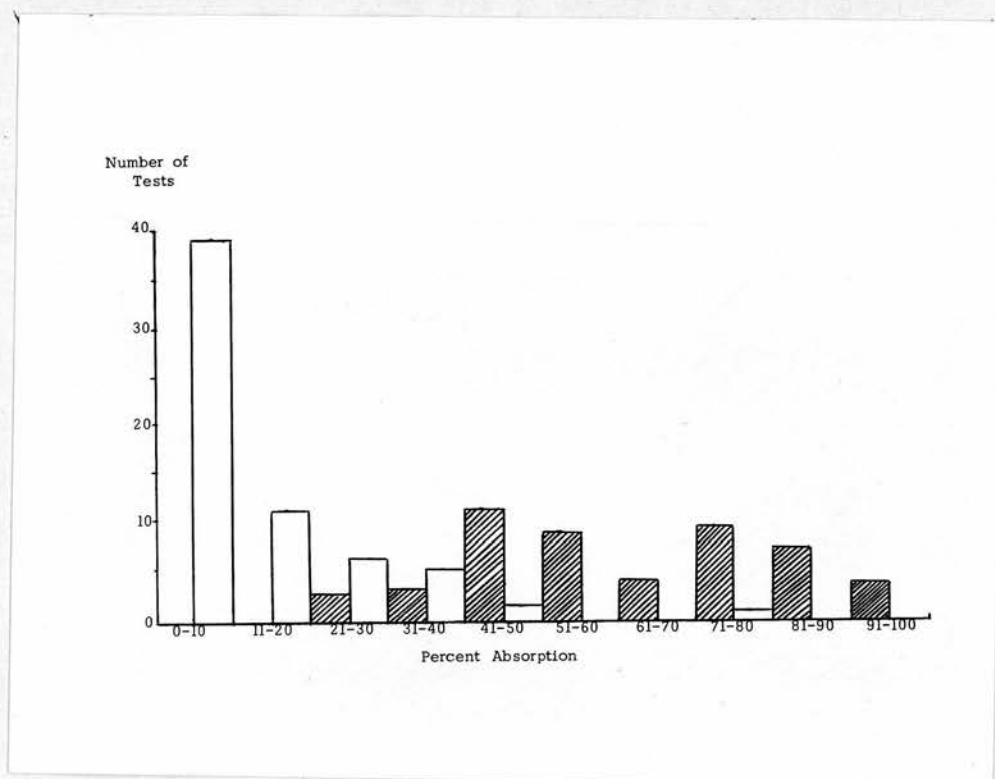
The mean absorption from 48 tests done on 24 control subjects was 64% (Standard Deviation \pm 19%), with a range from 30% to 98%. The mean absorption from 62 tests done on 31 subjects with pernicious anaemia was 11% (Standard Deviation \pm 15%), with a range of 0% to 80%. Five subjects with pernicious anaemia (16%) had at least one absorption test giving a value of greater than 30%; the paired test values in these particular subjects are shown in Table XIII. On only one occasion (3%) in all the pernicious anaemia subjects were values above 30% obtained on each of two occasions. The distribution of results in these 24 control and 31 pernicious anaemia subjects is shown in Fig. 21.

It may be noted for completeness that where the test dose was given with a vitamin B12-free meal, the lowest absorption in 11 control subjects was 47% and this value was exceeded on at least one occasion

TABLE XIII: *Results of absorption tests in four subjects with pernicious anaemia (PA) where at least one test gave an absorption value above 30% of the test dose*

PA Patient	Absorption (%)	
	Test I	Test II
1	33	19
2	39	80
3	38	10
4	36	12
5	22	42

FIG. 21: *Vitamin B12 absorption measured in the fasting state by whole body counting in 24 control subjects (48 tests) and 31 subjects with pernicious anaemia (62 tests)*



▨ = Control subjects

□ = Pernicious anaemia

(57%) by one of 11 pernicious anaemia subjects. That single subject absorbed 34% on another occasion. The distribution of results in these 11 control and 11 pernicious anaemia subjects is shown in Fig. 22.

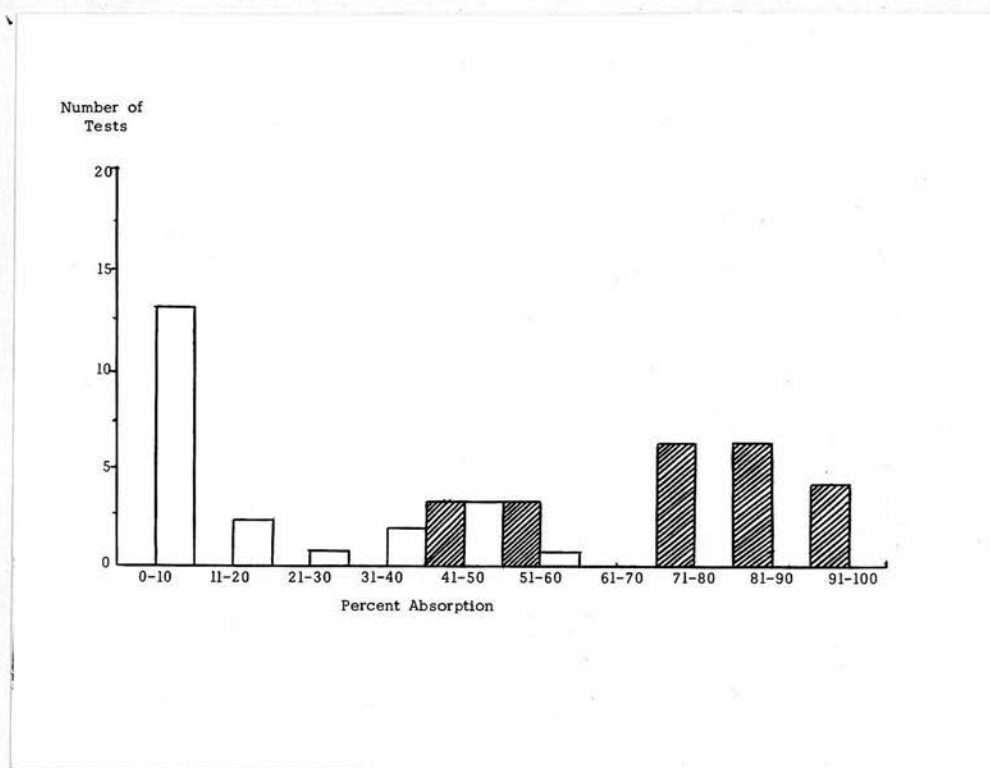
Reproducibility of Absorption Tests.

The subjects used in this part of the study are shown in Table XIV. Fig. 23 shows the relation between two absorption tests done in the fasting state. There was considerable variation in the results obtained, and this was especially true in the control and achlorhydric subjects. The least variation was found in the pernicious anaemia and malabsorption groups.

Fig. 24 shows that where the vitamin B12 was given with a vitamin B12-free meal, the overall correlation between the two tests was better. This improvement was a statistically significant one for control ($p < 0.01$) and for achlorhydric ($p < 0.05$) subjects, but not for subjects with pernicious anaemia or those who had had a partial gastrectomy. An insufficient number of subjects with malabsorption had been given tests with vitamin B12-free meals for a comparison to be made for these subjects.

The mean absorption and standard deviation for each subject group for each absorption test, done either with or without a vitamin B12-free meal, as well as the correlation coefficients for the paired tests and the probability estimate of the significance of these, are shown in Tables XV and XVI. The percentage differences between sequential paired tests are shown in Table XVII.

FIG. 22: *Vitamin B12 absorption measured with a vitamin B12-free meal by whole body counting in 11 control subjects (22 tests) and 11 subjects with pernicious anaemia (22 tests)*



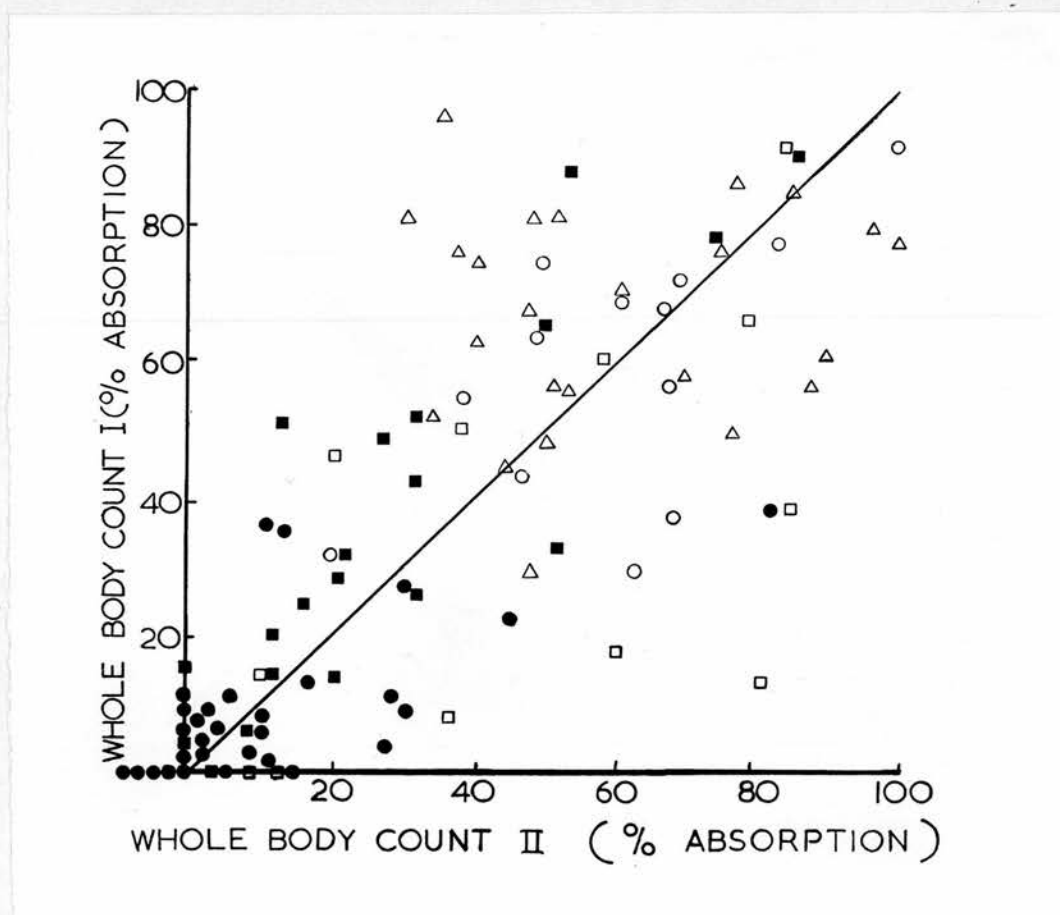
▨ = Control subjects

□ = Pernicious anaemia

TABLE XIV: Subjects used to study the reproducibility of Vitamin B12 absorption tests done by whole body counting

Test Conditions	Achlorhydria		Partial Gastrectomy	Malabsorption	Controls	Total
	Pernicious Anaemia	No Pernicious Anaemia				
Fasting	31	12	13	19	24	100
Food	11	6	7	2	11	37

FIG. 23: *Correlation between the first and second absorption tests in fasting subjects*



Δ = Control subject

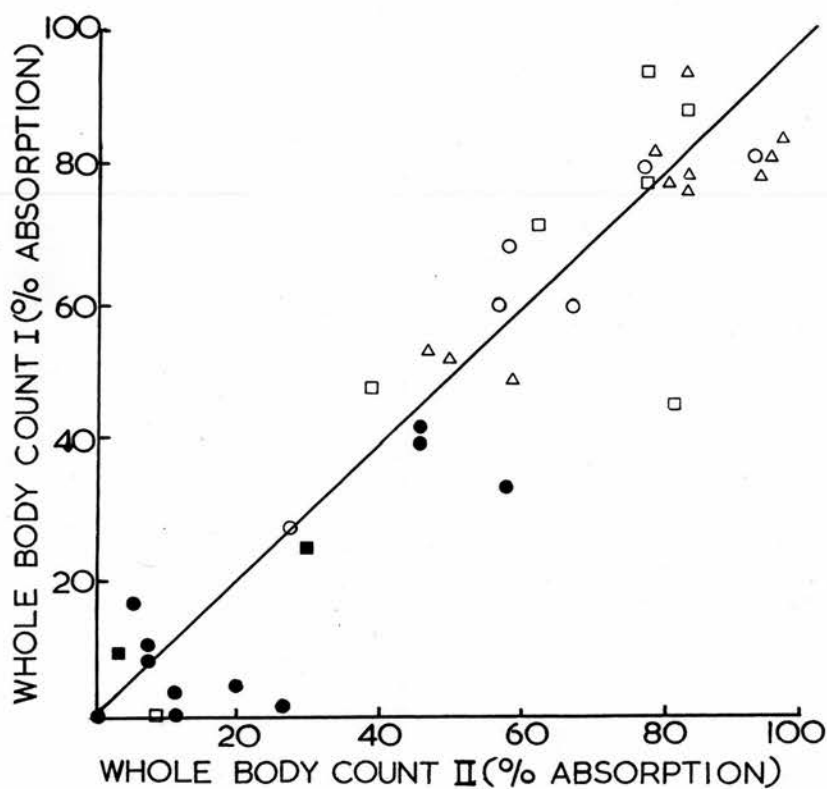
O = Achlorhydria

● = Pernicious anaemia

□ = Gastric operation

■ = Intestinal malabsorption

FIG. 24: *Correlation between the first and second absorption tests in subjects who had vitamin B12-free meals*



△ = Control subject

○ = Achlorhydria

● = Pernicious anaemia

□ = Gastric operation

■ = Intestinal malabsorption

TABLE XI: Mean absorption values and standard deviations (SD) for each test series and the correlation coefficients (r) and probability values (p) for test pairs where absorption tests were done in the fasting state

Group	Number	Whole Body Count I		Whole Body Count II		r	p
		Mean (%)	SD (%)	Mean (%)	SD (%)		
Controls	24	68	16	60	21	0.09	>0.1
Achlorhydria	12	61	18	64	18	0.35	>0.1
Pernicious anaemia	31	11	12 ¹	12	18 ¹	0.64	< 0.001
Gastrectomy	13	37	30	50	30	0.60	0.02
Malabsorption	19	39	26	29	23	0.86	< 0.001

¹Skewed distribution. Spearman rank correlation coefficient (Siegel 1956) test done.

TABLE XVII: Mean absorption values and standard deviations (SD) for each test series and the correlation coefficients (r) and probability values (p) for test pairs where absorption tests were done with a Vitamin B12-free meal

Group	Number	Whole Body Count I		Whole Body Count II		r	p
		Mean (%)	SD (%)	Mean (%)	SD (%)		
Controls	11	73	15	77	18	0.85	< 0.001
Achlorhydria	6	65	22	62	19	0.95	0.001
Pernicious anaemia	11	14	16 ¹	21	19 ¹	0.82	0.01
Gastrectomy	7	63	31	61	28	0.88	< 0.01
Malabsorption	2	-	-	-	-	-	-

¹Skewed distribution. Spearman rank correlation coefficient (Siegel 1956) test done.

TABLE XVII: The percentage differences between two sequential vitamin B12 absorption tests done in the fasting state and with a vitamin B12-free meal

Group	Differences (%) Between Paired Tests				
	Fasting		Food		
	Mean	Range	Mean	Range	
Control	21	0-61	8	2-15	
Achlorhydric	15	1-41	6	0-10	
Pernicious anaemia	9	0-41	9	0-24	
Gastrectomy	22	4-46	11	2-26	
Malabsorption	13	2-37	-	-	

The mean absorptions of vitamin B12 were greater when the tests were done with a vitamin B12-free meal in all groups. The increase was greatest in the gastrectomised subjects (Tables XV and XVI). As the groups are small, unequal in numbers, and represent different subjects, the significance of these differences is difficult to assess.

Comparison of Results Obtained Using Two Machines Simultaneously.

The variation in the counting-rates on the day of administration (100% value) showed no consistent or systematic trend with either 58Co vitamin B12 or 57Co vitamin B12. The results were expressed, therefore, for convenience as coefficients of variation. These are shown in Table XVIII. There was no significant difference ($p > 0.1$) between the two monitors.

The absorption values on the seventh day obtained in both monitors are shown in Fig. 25. The regression equation was $y = 1.068x + 0.819$, where y is the % absorption found in the S.R.R.C. monitor and x is that in the R.I.E. II monitor. The correlation coefficient of 0.976 was highly significant, while the regression coefficient did not differ significantly ($p > 0.1$) from the expected value of 1.0 and the intercept was not significantly different ($p > 0.05$) from the expected value of zero. Typical subject and background counting-rates are given in Table XIX.

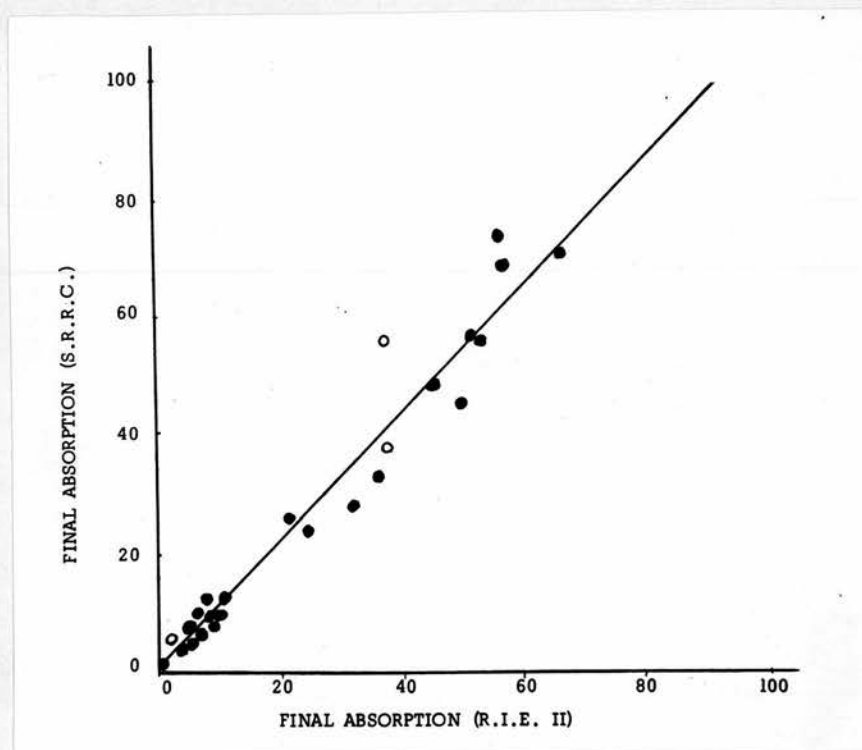
The results obtained in this part of the study therefore indicate that conflicting results obtained in different centers are not likely to be the result of machine variation.

TABLE XVIII: *Summary of variations in the counting rate up to 6 hours after administration of an oral dose of ^{58}Co Vitamin B12 or ^{57}Co Vitamin B12. Results shown as coefficients of variation.*

Isotope	Energy Range (Mev)	Number of Studies	Standard Deviations of 100% Values					
			S.R.R.C.			R.I.E. II		
			Min.	Max.	Mean	Min.	Max.	Mean
^{58}Co	0.20-1.00	16	0.7	8.6	4.1	0.6	9.9	3.4
^{58}Co	0.66-1.00	16	1.2	10.3	5.3	0.7	12.9	3.9
^{57}Co	0.17-0.30	13	3.6	11.6	7.1	3.0	15.1	7.1

In a further patient who was monitored only twice in each monitor after receiving ^{58}Co Vitamin B12, the range was 3.2% in the S.R.R.C. monitor and 8.9% in the R.I.E. II monitor.

FIG. 25: Comparison of vitamin B12 absorption values obtained using two machines simultaneously (correlation coefficient = 0.98)



● = 58Co

○ = 57Co

TABLE XIX: *Mean background counting rates in the
R.I.E. II and S.R.R.C. monitors and
typical subject counting rates per uc*

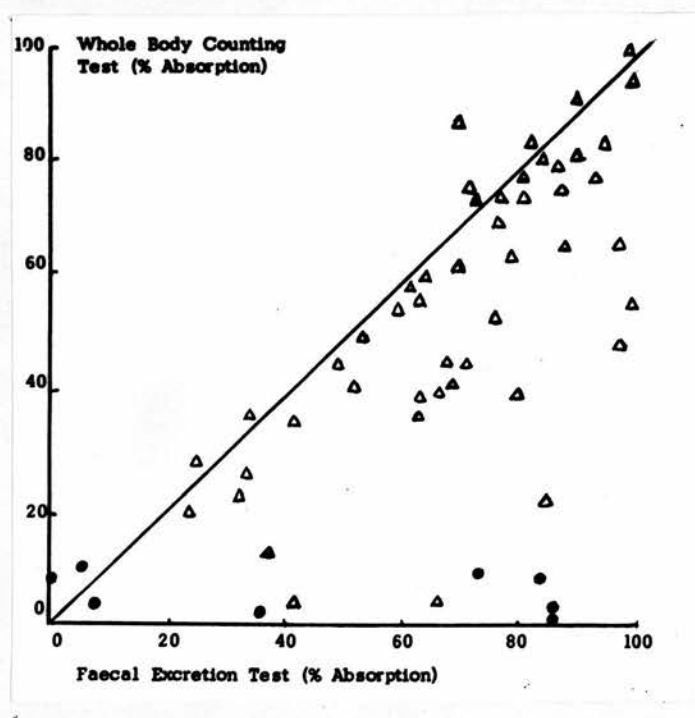
Energy Range (Mev)	0.66 - 1.00	0.20 - 1.00	0.03 - 0.17
R.I.E. II background, c.p.m.	250	1300	875
S.R.R.C. background, c.p.m.	255	1140	620
R.I.E. II subject c.p.m./uc	1400	5400	4000
S.R.R.C. subject c.p.m./uc	8400	26,000	9600

Correlation of Vitamin B12 Absorption Measured by Whole Body
Counting and by Calculating from the Faecal Excretion of
Radioactivity.

In 56 subjects, vitamin B12 absorption was measured by whole body counting and by the faecal excretion method simultaneously. The results are shown in Fig. 26, and the line of identity for the test results has been drawn to show the ideal situation for these paired results.

Each patient was given careful instructions about the importance of a complete collection, but no patient has been excluded from the study on the basis of an unexpected result. In a number of instances, a much higher retention is shown by faecal measurements compared with the whole body counter result. On the other hand, of the ten instances in which a greater retention is shown by whole body counting than by faecal measurement, in only one case was the difference greater than 8%. It would seem that the much larger discrepancies below the line of identity (Fig. 26) are due to incomplete stool collections. This is supported by the fact that 8 patients with proven pernicious anaemia showed the expected low absorption on whole body counting (all had absorption of less than 10%), but only 3 on faecal collection. From Fig. 26 it would seem that in at least 13 of the 56 subjects (23.2%) an incomplete collection of faeces was made.

FIG. 26: *Correlation between vitamin B12 absorption measured simultaneously by whole body counting and by the faecal excretion test*



Δ = Non-pernicious anaemia

\bullet = Pernicious anaemia

CLINICAL STUDIES USING WHOLE BODY COUNTING

In this part of the work a study was made of the relation between intrinsic factor secretion and vitamin B12 absorption. As far more intrinsic factor is secreted under normal circumstances than is required for vitamin B12 absorption, subjects who were likely to have a limited intrinsic factor secretory capacity, by virtue of having pernicious anaemia, being achlorhydric, or having had a gastric resection, were studied. In this study tests of intrinsic factor secretion in response to histamine or pentagastrin were done during the period when vitamin B12 absorption was being measured. The concentration of intrinsic factor in the gastric juice secreted within one hour of stimulation was measured, and the total intrinsic factor secreted during that hour was calculated from the volume of gastric juice secreted; the gastric juice volumes secreted by subjects with pernicious anaemia and achlorhydria are shown in Fig. 27. In comparing intrinsic factor secretion and vitamin B12 absorption, no difference was found dependent on whether intrinsic factor concentration or the total output of intrinsic factor in the hour following stimulation were used. The results given therefore refer to total intrinsic factor secreted in the hour after gastric stimulation.

Fig. 28 shows the relation found between vitamin B12 absorption tests done in the fasting state and intrinsic factor secretion. In most cases two tests of absorption were done, and these are both shown. In general, subjects with greater intrinsic factor secretory

FIG. 27: Gastric juice volumes in the hour following stimulation with histamine or pentagastrin

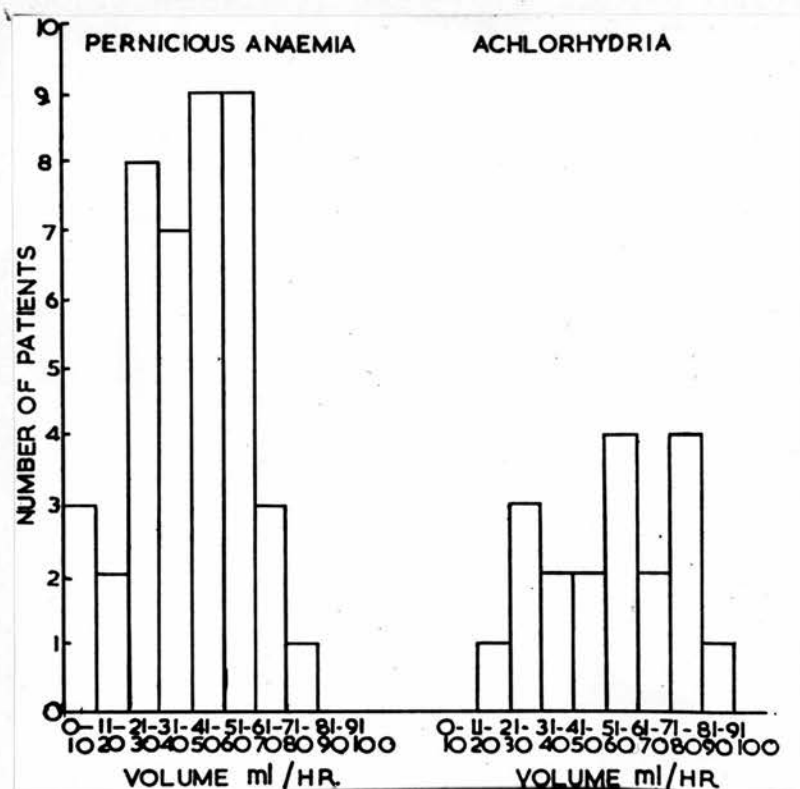
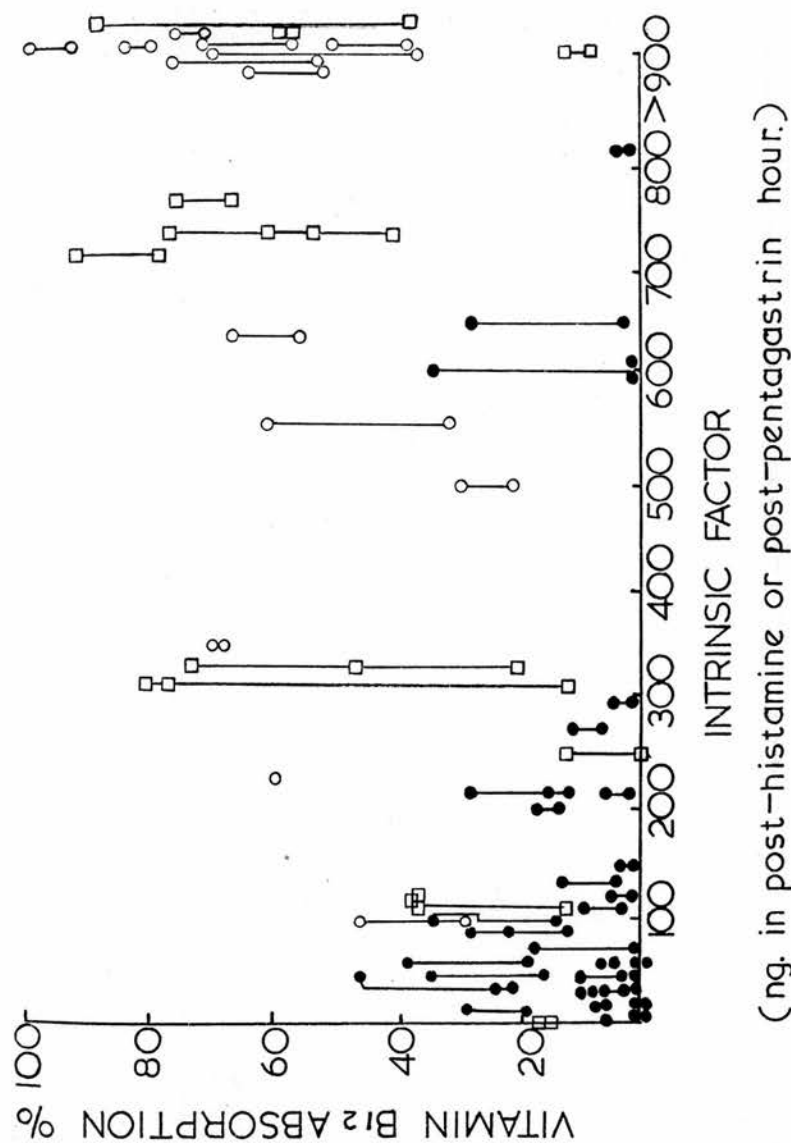


FIG. 28: The relationship between the absorption of vitamin B12 in the fasting state as determined by whole body counting and intrinsic factor production



○ = Achlorhydria
 □ = Achlorhydric partial gastrectomy
 ● = Pernicious anaemia

capacity absorbed more vitamin B12. This can be illustrated by comparing the highest or lowest absorption value for each individual with the intrinsic factor secretion; as illustrated in Table XX, χ^2 tests based on divisions at 35% of vitamin B12 absorption and an intrinsic factor secretion level of 300ng units per hour show that those with intrinsic factor secretions above 300ng units per hour absorbed more than 35% of the test dose significantly more often. Nevertheless, overlapping of values occurred, and it was not possible to separate absolutely subjects with pernicious anaemia from those with achlorhydria.

Fig. 29 shows the relation between vitamin B12 absorption and intrinsic factor secretion where the vitamin B12 test dose was taken with food. In this situation the vitamin B12 test dose might be retained in the stomach longer, allowing more time for its union with intrinsic factor, and in addition, the food might stimulate greater intrinsic factor secretion. There are many fewer subjects for analysis here, but it can be seen that there is still no clear separation between those with pernicious anaemia and those with achlorhydria.

The data obtained in this part of the study indicated overall that, at least when the test dose was given in the fasting state, those who could secrete more intrinsic factor tended to absorb more vitamin B12. However, there were certain subjects in whom absorption was variable, or in whom anomalous absorption seemed to have occurred. These will be described in more detail.

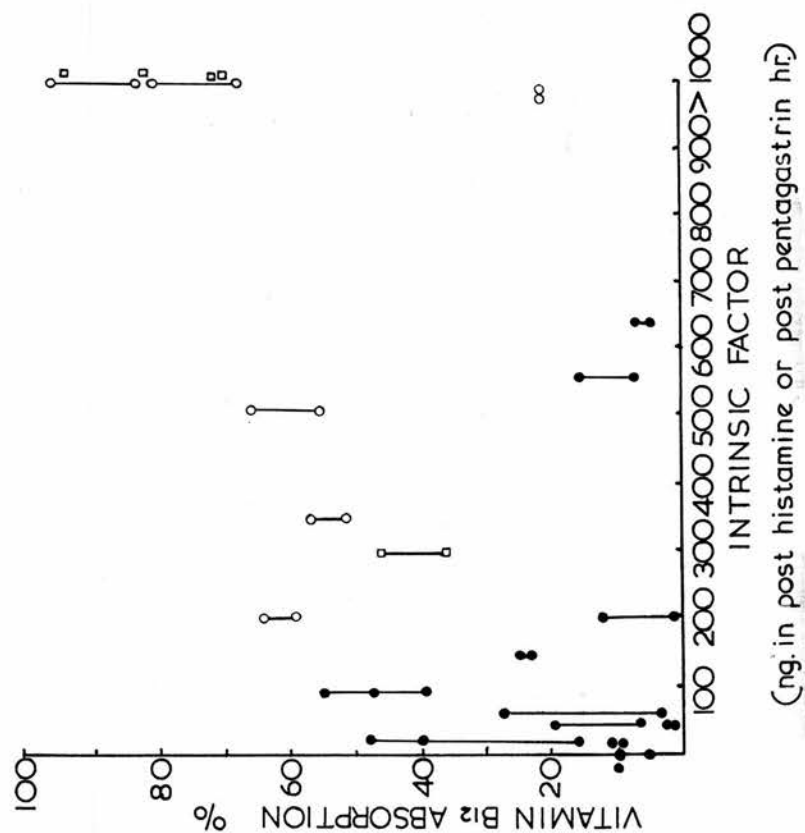
TABLE XX: *Vitamin B12 absorption and intrinsic factor secretion*

		Intrinsic Factor Secretion (ng units/hour)		
		≤ 300	> 300	
Maximum Vitamin B12 Absorption (%)	> 35	5	19	$\chi^2 = 21.0$
	≤ 35	26	4	$p = < 0.001$

		Intrinsic Factor Secretion (ng units/hour)		
		≤ 300	> 300	
Minimum Vitamin B12 Absorption (%)	> 35	1	15	$\chi^2 = 21.4$
	≤ 35	30	8	$p = < 0.001$

p = probability of a chance association between the observations made.

FIG. 29: The relationship between the absorption of vitamin B12 (whole body counting) when given with a meal and intrinsic factor production



0 = Achlorhydria
 □ = Achlorhydric partial gastrectomy
 ○ = Pernicious anaemia

Variable Vitamin B12 Absorption: In subjects who had had a partial gastrectomy or who had malabsorptive disease, two absorption tests done during successive weeks could give results in both the normal absorption range and the malabsorption range. When the tests were done in the fasting state, 4 of 13 subjects who had a partial gastrectomy gave one result above the lowest value in a control (30%) and one below. In 3 of these 4 subjects one absorption value was above 40% and the other below 20%. Five of 19 subjects with malabsorptive disease gave similarly conflicting results, though in this instance, in four of the subjects, one or both of the results could be considered as borderline, only one giving a gross disparity (51% and 14%). Fewer results were available where the tests had both been done with a vitamin B12-free meal, but it was found that one of 7 subjects who had a partial gastrectomy gave conflicting results.

Table XXI shows two achlorhydric subjects in whom variable absorption values occurred. One was found to be achlorhydric incidentally, his general health did not alter during the test period, and he received no treatment. On two occasions tests in the fasting state gave normal values, and on two occasions tests with food gave values in the malabsorption range. In general, food was not found to inhibit vitamin B12 absorption in this study.

The second subject suffered from iron deficiency anaemia of unknown cause, was achlorhydric, and had a low intrinsic factor secretion before and after treatment for iron deficiency. Before

TABLE XXI: Variability of Vitamin B12 Absorption (%)
(2 cases)

Diagnosis	Intrinsic Factor (ng/hr)	Serum Vitamin B12 (pg/ml)	Serum Folate (ng/ml)	Vitamin B12 Absorption		
				Fasting	Food	
Achlorhydria	3500	201	6.2	55	39	27
Idiopathic iron-deficiency anaemia	490 ¹	344	5.6	31	21	65 ² 55 ²

¹ Before and after treatment.

² After treatment.

treatment vitamin B12 absorption in the fasting state was low. After treatment vitamin B12 absorption with food was very much higher.

It should be noted that absorption values in achlorhydric subjects were generally normal. When two sequential tests were done, all but one of the results in 12 subjects tested in the fasting state were within the normal range. These tests included those of the achlorhydric subjects reported above. When the tests were done with food, only one of 6 subjects, the first achlorhydric subject reported above, fell below the normal range.

Deficient Intrinsic Factor Secretion with Normal Vitamin B12

Absorption: Table XXII shows two subjects whose intrinsic factor secretory capacity was very low, being well within the range of most patients with pernicious anaemia, but who absorbed vitamin B12 normally. In both cases the gastric function test was done twice, and in the second a barium meal did not show any gastric deformity likely to have interfered with the collection of gastric juice. Both were found to be achlorhydric incidentally. Both had normal serum folate levels. The first subject had a low serum vitamin B12 level in spite of normal vitamin B12 absorption on three occasions by whole body counting, and once by the Schilling test. The second subject had a low/normal serum vitamin B12 level and three normal vitamin B12 absorption tests by whole body counting where the test

TABLE XXII: Vitamin B12 Absorption with Intrinsic Factor Deficiency
(2 cases)

Diagnosis	Intrinsic Factor (ng/hr)	Vitamin B12 Status				Serum Folate (ng/ml)	
		Whole Body Count Absorption %		Schilling Absorption %	Serum Vitamin B12 (pg/ml)		
Achlorhydria	80	43	46	31	12.4	79	16.4
Achlorhydria	85	39 ¹	46 ¹	53 ¹	-	149	3.2 / 6.2

¹Test dose given with food.

doses had been given with food. In the third absorption test (53%), the final count was delayed for two weeks, the passage of stools was checked by history taking, and laxatives were given to ensure that all unabsorbed radioactivity had been eliminated.

Pernicious Anaemia with Relatively High Intrinsic Factor

Secretion: Table XXIII shows three achlorhydric subjects, all of whom had pernicious anaemia with serum parietal cell auto-antibodies; each had malabsorption of vitamin B12 as measured by whole body counting, which could be reversed with exogenous intrinsic factor as measured by the Schilling test. No gross small intestinal abnormality was found, and all responded to parenteral cyanocobalamin therapy. Each had an intrinsic factor secretory capacity sufficient to allow normal vitamin B12 absorption in other subjects (Figs. 2 and 28).

TABLE XXIII: Relatively high intrinsic factor secretion
in pernicious anaemia (3 cases)

IF ¹ (ng/hr)	Vitamin B12 Status					Serum Folate (ng/ml)	Small Intestine		Autoanti- bodies	
	Whole Body Count		Absorption (%)		Schilling Test with IF (%)		Serum Vitamin B12 (pg/ml)			
			After 2 Tetracycline							
548	15	5	6	15	50	12.8	normal	normal	+++	-
825	1	2	-	17	50	-	normal	-	+	-
650	8	7	11	13	100	15.0	² duodenal diver- ticula	normal	+	-

¹Intrinsic factor.

²250 mg four times daily for four days.

³Parietal cells.

DISCUSSION

WHOLE BODY COUNTING IN THE MEASUREMENT OF VITAMIN B12 ABSORPTION

All of the established vitamin B12 absorption tests described above are satisfactory for routine clinical purposes, provided their limitations are kept in mind. In the majority of cases, they will distinguish between those with and without pernicious anaemia and will demonstrate the effect of oral intrinsic factor in that disease. However, with the exception of the faecal excretion test and the whole body counting test, they give only qualitative or at best semi-quantitative results and are therefore unsuitable for a close analysis of problems of vitamin B12 absorption. It has been claimed (Weissberg and Glass 1966) that the double isotope modification of the hepatic uptake test gives quantitative results. But this test depends on certain assumptions regarding the metabolism in the body of orally and intravenously administered vitamin B12, it requires the introduction of correction factors to allow for the varying counting efficiencies of the two different isotopes used, and it may be subject to errors resulting from natural variations in liver geometry from subject to subject, to say nothing of the effects of liver disease. Finally, it is unsuitable for repeated studies on the same subject as a result of the marked variations in baseline radioactivity of the liver which occur under these circumstances. In short, though it is an excellent semi-quantitative test, it has not yet been confirmed

that the hepatic uptake test gives good quantitative measurements. On the other hand, in spite of some reservations discussed by Glass (1963), most workers regard the faecal excretion test as being quantitative. Provided that all unabsorbed radioactivity is collected as it is passed in the faeces, the absorption from the test dose can be simply, if indirectly, estimated. The problem, of course, is that the unaesthetic nature of the test results in a frequent failure to collect all the faeces passed. Ganatra et al. (1965) and Campbell and Craswell (1970) have introduced double isotope faecal excretion tests to overcome this problem, at least in part, but in so doing the original test loses some of its simplicity by the addition of another variable.

The use of whole body counting goes a long way to meeting the requirements for a conceptually simple test which will measure vitamin B12 absorption directly and quantitatively, and yet be aesthetically acceptable to the subject. Machines such as those used in this study are becoming increasingly available, and they have a high degree of sensitivity. The sensitivity of the machines R.I.E. I and R.I.E. II was of the order of 1.4%. In addition, the machine comparison study, reported above, indicated that where broadly similar machines are used, variation between these should not materially affect results. The machine itself is therefore a very minor source of error.

Nevertheless, there are potentially important sources of error. The first of these involves the determination of the 100% value. As

discussed above, this can be reduced at the outset by selecting a suitable geometry for counting. In the case of the vitamin B12 isotopes, the scanning bed geometry used in this study is probably the best available. Using ^{58}Co as a label, the mean coefficient of variation of the 100% count was $\pm 3.2\%$ in the 0.2-1.0 Mev energy range. This would mean that a vitamin B12 absorption of 30% could be 29% or 31%; on the other hand, in individual cases higher coefficients of variation were found, and where this was 10%, for example, a final 30% value could lie anywhere between 26% and 35%. The 100% error would be somewhat greater where ^{57}Co was used, as the mean coefficient of variation with this isotope was 6.9%. Overall, the coefficients of variation of the 100% value in this study matched favourably with figures given by other workers--for ^{58}Co , $\pm 3\%$ (Warner and Oliver 1966), $\pm 10\%$ (Tappin et al. 1966), $\pm 3.4\%$ (Boddy et al. 1969), and for ^{57}Co , $\pm 2.7\%$ (Boddy et al. 1969), $\pm 13\%$ (Naversten et al. 1969)--though it was not found that the variation fell after about three hours as reported by Tappin et al. (1966). Furthermore, direct comparison of the 100% values for ^{57}Co and ^{58}Co between the R.I.E. II machine and that used by Boddy et al. (machine S.R.R.C.) showed no significant differences in the coefficients of variation. Overall, therefore, in the majority of cases the error in the final absorption value due to the 100% value should be quite low.

One disadvantage of this test is that seven days elapse

between giving the vitamin B12 test dose and taking the final count from which the absorption is calculated. During this time some of the absorbed vitamin B12 could be excreted in the bile, of which about a quarter to a third could reach the faeces (Grasbeck et al. 1958), and some could be lost in the urine. The first factor was not assessed in this study. Grasbeck et al. (1958) showed, however, that when a 0.5 μ g test dose was given parenterally, after an equilibration period of four days had passed, about 1.1 (\pm 0.3)% of the total radioactive vitamin B12 in the body passed into the bile daily. If it were assumed that only this amount were passing into the bile daily after an oral dose, then, assuming 50% absorption in a normal subject, about 2.5-5.0% of the original test dose would return to the gut over seven days; this would amount to an error of about 0.8-1.7% if one-third were excreted in the faeces. The loss of radioactivity into the urine over the one week period of the test is probably rarely greater than 1-2% of the test dose as judged by the results of this study. Occasional subjects may, however, lose up to 5% during this period for no obvious reason. In summary, granted a number of assumptions for biliary excretion, the combined negative error from urine and bile losses of unabsorbed radioactive vitamin B12 is unlikely to exceed 4% of the test dose in any subject, and is probably less than half of this on most occasions.

The reason for the seven day delay before taking the final count is, of course, to allow time for the faecal excretion of unabsorbed radioactive material. Failure of excretion of such unab-

sorbed material in effect constitutes the most important potential error in the method. The problem is to choose a time giving a reasonable period for the excretion of unabsorbed radioactivity, while allowing the test to be completed within a reasonable period of time. The investigations done in this study suggest that by the end of a week few subjects are excreting much radioactivity in the stool, the mean excretion on the seventh day being 0.1% of the test dose. This agrees with the data of other workers. Naversten et al. (1969) suggested an average decrease in whole body radioactivity of 1.4% between the seventh and fourteenth days in their recommended optimal energy range for counting; Reizenstein et al. (1961) suggested a mean decrease of 1.1% between the seventh and twenty-second days; and Reizenstein and Nyberg (1959) reported that only 0.3-0.5% of the test dose was excreted on the eighth day of the test. It may be (Grasbeck et al. 1958) that if there was too long a delay in taking the final count, a negative error from continued biliary loss could occur. Clearly it would be useful to have some way of detecting unabsorbed and unexcreted radioactivity simply. The testing for radioactivity of at least the last stool passed before the final whole body count would be one possibility. This, however, reintroduces an unaesthetic aspect to the test for the subject, and invites precisely those inaccuracies of collection the whole body counting test seeks to avoid. Alternatively, a largely non-absorbed and easily visible marker, such as carmine, could be given with, or

shortly after, the vitamin B12 test dose and counting delayed until all colour had disappeared from the stool. This was not done in the study reported here, but it would have to be shown by faecal excretion experiments that radioactive vitamin B12 and carmine were excreted in a similar method. Profile scanning at the time of the final count is a very simple procedure which requires no patient cooperation, and the results obtained in this study indicate that it may be a useful way of checking for unabsorbed and unexcreted radioactivity. While the full validation of the method would require further studies, those obtained indicate that where an "intestinal" peak can be seen on the scan, excretion of unabsorbed radioactivity has not been complete. In spite of the total oral dose of radioactivity being quite small (1.0 μ c), even in the presence of a substantial "liver peak" indicating normal absorption, 10% of the test dose can be detected in the rectosigmoid part of the large bowel. Less (5%) could be detected where the "liver peak" was small, as in malabsorption. A note of caution has to be introduced, however, where a single peak scan is seen. This cannot be accepted as unequivocal evidence that all unabsorbed radioactivity has been excreted, as such a pattern was seen throughout the week of an absorption test in 1 of 15 control subjects with normal absorption and 2 of 9 subjects with vitamin B12 malabsorption. If the results in the sample of 24 subjects studied in this work are generally representative, then 7% of subjects with normal vitamin B12 absorption and

22% with vitamin B12 malabsorption will show only single peak scans during the week of the test. In summary, therefore, at the present stage of its development, profile scanning should only be used to detect definite failure to eliminate unabsorbed radioactivity.

Summary.

Only the faecal excretion and whole body counting tests of vitamin B12 absorption can be considered as quantitative. In the latter, the whole body counter itself has a high degree of sensitivity, and variation between machines is probably not a serious source of error. The most important sources of error arise in determining the 100% count, where mean coefficients of variation range from 3% to 13%, being somewhat higher for ^{57}Co than ^{58}Co , from losses due to urinary and biliary excretion of absorbed vitamin which are probably not more than 4% of the test dose, and from failure of excretion of unabsorbed vitamin which is probably not a serious error in most subjects counted no earlier than the seventh day. This last error can be minimized by giving a laxative during the test, and profile scanning at the time of the final count may help in detecting subjects where unabsorbed vitamin has not been excreted.

THE RESULTS OF VITAMIN B12 ABSORPTION TESTS PERFORMED BY WHOLE BODY COUNTING

Control and Achlorhydric Subjects.

The range of vitamin B12 absorption in control subjects is very wide, being 30-98% (mean value 64% [S.D. ± 19]) in the work de-

scribed here. These results are in keeping with the reports of others (Table III, Introduction), where absorption values range from 21% to 98% with test doses of 0.5-1.1 μ g of vitamin B12. The results of Irvine et al. (1970) are virtually identical to those reported above; this can readily be accounted for as these workers utilised the machine R.I.E. II, used in this study, as well as the method developed and reported on above. In addition to the wide range of normal absorption, however, the performance of sequential tests over a two week period showed that considerable variation between the two absorption tests could occur (Fig. 23). This resulted in a poor correlation between the test pairs (Table XV), with a mean difference between the two absorption tests of 21% (Table XVII). Studies on the reproducibility of vitamin B12 absorption using the whole body counting method have not been reported previously, but studies using the faecal excretion method have shown absorption differences between sequential tests of up to 19% (Halsted et al. 1956) and 48% (Meyer et al. 1955). Mean variation in both these studies was less than in the present one, being 7% (Halsted et al. 1956) and 9.3% (Meyer et al. 1955).

Vitamin B12 absorption in the fasting state in subjects who were achlorhydric, but who did not have pernicious anaemia, did not differ significantly from normal (Table XV). Only one such achlorhydric patient had an absorption below the lowest limit of normal, and then on one occasion only (Case 2, Table XXI). These results

agree closely with those of Irvine et al. (1970) and show that absorption studies with this method will usually differentiate between achlorhydric subjects with and without pernicious anaemia. As with the control subjects, the correlation between sequential tests was not good (Table XV), though the mean difference between the two tests was less, being 15% (Table XVII).

The reason for these differences in absorption in the same subject are not known, and are often attributed to poor reproducibility of the method being used (Glass 1963, Townsend et al. 1968). In the faecal excretion test, as some 20-30% of stool collections may be incomplete (Callender et al. 1966), this would be a good reason. However, as the evidence discussed above suggests that marked retention of unabsorbed radioactivity after a week is probably unusual, and in this study all patients were given a laxative on the third day of the test to encourage elimination, the high incidence of variability may be due to variable absorption rather than to methodological factors. This view is supported by the observation that variation in absorption test results was much less in subjects with malabsorption or pernicious anaemia (Table XI), where the capacity for variation is low. If failure of excretion of unabsorbed vitamin B₁₂ was a major factor in producing the variation in results, greater variation in these groups might have been expected. It may be that variable results in subjects capable of normal absorption depends in large part on how much intrinsic factor happens to be present in the stomach when the test dose is given.

Pernicious Anaemia.

The mean absorption of vitamin B12 in pernicious anaemia in the present study was 11.0% (S.D. $\pm 15.0\%$); this mean value is comparable to those found by others (Tables III and IV, Introduction) which range from 3.3% (Bozian et al. 1963) to 13.1% (Meyer et al. 1968). In studies by other authors, little overlap in absorption values has been found between subjects with pernicious anaemia and control subjects, though this has occasionally occurred (Tables III and IV, Introduction).

An analysis of cases in which the absorption test results overlapped with the control range was done. In one case (Tappin et al. 1966) the authors suggested that unabsorbed radioactive vitamin B12 was still in the bowel; this seemed a good explanation, as a urinary excretion test done simultaneously gave a value of only 3.0% (author's lowest limit of normal 7.0%). In the report of Irvine et al. (1970) 8 of 46 subjects (17.0%) with pernicious anaemia, 6 of 73 (8.0%) with achlorhydria, and 2 of 73 (3.0%) control subjects had absorption values between 21.0% and 25.0%, which seemed to constitute an equivocal range between normal absorption and malabsorption. Meyer et al. (1968) did not give a range of normal absorption, but 2 of their 8 cases listed as having pernicious anaemia gave results well above the other six (28.5%, 32.9%); unfortunately, the definition of pernicious anaemia given by these authors is very unclear, and as neither of these patients showed

significant augmented absorption when the test was repeated with intrinsic factor (27.5%, 39.0%), they may not have had pernicious anaemia at all. Thus, the only instances in which an unaccounted for overlap between pernicious anaemia, defined by clear diagnostic criteria, and control or achlorhydric subjects has occurred have been those of Irvine et al. (1970). These overlapping results were within a very small range.

In the present series, in which the lowest absorption of vitamin B₁₂ by a control was 30.0%, 5 subjects designated as having pernicious anaemia exceeded this value in at least one of two tests (Table XIII), and in two further cases a value of 30.0% was recorded in one of two tests. Only one of these 7 subjects absorbed 30.0% or more of the test dose on both test occasions; in the other 6 subjects, the lower of the two absorption values ranged from 10.0% to 27.0%. One subject absorbed 39.0% and 80.0% on the two tests.

The first possibility to explain these anomalous results is that unabsorbed radioactivity had not been excreted. This would imply failure of excretion in 13.0% of the 62 tests carried out, in spite of the use of a laxative, which seems a high failure rate in view of the results of the radioactivity excretion studies reported above, which were carried out during actual absorption tests. In those studies, only one of 45 (2.2%) subjects excreted more than 1.5% of the test dose on the seventh day of the test.

In addition, none of the pernicious anaemia subjects with absorption values of 30.0% or more were recorded as having been constipated during the test. Unfortunately, none were recounted after seven days.

Another possibility is that the original diagnosis of pernicious anaemia was wrong in these cases. All subjects were designated as having pernicious anaemia because they had a megaloblastic marrow with low serum vitamin B12 levels and vitamin B12 malabsorption corrected with exogenous intrinsic factor as assessed by the urinary excretion test. The marrow and serum vitamin B12 levels are unlikely to be wrong, as all marrow films were examined by very experienced haematologists and the vitamin B12 levels were done in a research laboratory in duplicate with control sera known to have normal or low vitamin B12 levels.

The urinary excretion test of vitamin B12 absorption is, of course, subject to errors due to incomplete collections, and the loss of even a small volume during the period of peak vitamin B12 excretion on the first test, where oral intrinsic factor was not given, could give a result indicating malabsorption. Failure to give the "flushing" dose of nonradioactive vitamin B12 would have the same results. The subsequent test with added oral intrinsic factor would then give a normal result, suggesting a diagnosis of pernicious anaemia.

As all these pernicious anaemia subjects had intact

stomachs, by definition, an alternative cause for vitamin B12 deficiency would have to be found. Prolonged dietary vitamin B12 deficiency, though rare in Britain, could be one cause; however, none of the pernicious anaemia subjects in this study had an obviously deficient diet. Adams and Cartwright (1963), using the urinary excretion test, have shown that gross variations occur in the results of vitamin B12 absorption tests in intestinal malabsorptive disease, so that both normal and abnormal results may be obtained in the same subject. Possibly, therefore, some of the subjects studied here had intestinal disease and by chance gave an abnormal, followed by a normal result unrelated to the giving of oral intrinsic factor. However, although all the subjects with malabsorption studied by Adams and Cartwright (1963) had megaloblastic anaemia, only one (Case M1) had unequivocal vitamin B12 deficiency, and that had been contributed to by a grossly deficient diet. It seems unlikely, then, that malabsorptive disease was a cause of many of the anomalous results in this work, though in the absence of intestinal studies, an ileal lesion, such as Crohn's disease, cannot be excluded.

A final possibility for hypothetical consideration would be that some of the anomalous patients described above did have pernicious anaemia and did, at least on a single occasion, absorb a normal amount of vitamin B12 from a small test dose. It may

be important that the absorption tests were carried out with crystalline cyanocobalamin, which is not the food form of vitamin B12. Heyssel et al. (1966) have shown that natural dietary vitamin B12 was as available for absorption as crystalline cyanocobalamin, at least in normal young adults. It is interesting to note, however, that normal older adults absorbed less of the natural dietary vitamin B12, and that there was some overlap in absorption values between this group and patients with pernicious anaemia. The number of subjects studied by these workers was small, but the results suggested that the ability to absorb dietary vitamin B12 may normally diminish with age; whether this also applies to cyanocobalamin was not investigated, but other evidence suggests that it may not (Glass et al. 1956, Chow et al. 1956). Finally, there is some evidence that deoxyadenosylcobalamin may be absorbed more slowly (Lee and Glass 1961) and possibly with greater difficulty (Heinrich and Gabbe 1964) than cyanocobalamin, although evidence on the latter point is conflicting (Lee and Glass 1961, Herbert and Sullivan 1964).

It has been stated (Ardeman and Chanarin 1965), that 500ng of intrinsic factor are needed to effect normal absorption from a 0.5µg dose of vitamin B12, and, as discussed below, at least a few subjects with pernicious anaemia can produce around that amount during histamine or pentagastrin stimulation. It is conceivable, therefore, when these evidences are put together, that occasionally

a subject with pernicious anaemia may accumulate sufficient intrinsic factor in the stomach overnight to allow a reasonable absorption from a small vitamin B12 test dose the following morning. The neutral pH and the low pepsin secretion in these subjects could allow prolonged survival of any intrinsic factor secreted.

It has to be admitted, however, that the vast number of vitamin B12 absorption tests in subjects with pernicious anaemia reported in the literature would suggest that if the above occurs, it must be relatively rare. Nonetheless, before effective therapy for pernicious anaemia became available, it was well recognised that spontaneous remissions could occur in this disease, and that such remissions could include a striking rise in the peripheral red blood cell count (Chanarin 1969). These remissions could conceivably have been the result of a transient increase in vitamin B12 absorption. Heyssel et al. (1966) have suggested that maximal absolute absorption of natural vitamin B12 in normal young adults occurs from a single intake of 3.0 μ g, that such an intake does not interfere with the absorption of a similar intake 4-6 hours later, and that a normal diet could provide three such intakes daily. A subject with pernicious anaemia would only have to absorb 10% from the first 3.0 μ g intake of the day and 5% from each of two subsequent intakes to acquire 0.6 μ g in a day; Heyssel et al. (1966) estimated that the minimal daily vitamin B12 intake needed to maintain health in man was 0.6-1.2 μ g.

Overall, in subjects considered to have pernicious anaemia, vitamin B12 absorption tests by whole body counting give results clearly below the lowest limit of normal in the great majority of cases. Occasional results within the normal range are unusual and are probably due to the inadequate excretion of unabsorbed radioactive vitamin B12 in most cases. In a few cases the diagnosis of pernicious anaemia may be wrong, and it is theoretically possible that a subject with pernicious anaemia might occasionally absorb an amount within the normal range.

Partial Gastrectomy and Intestinal Malabsorptive Disease.

Adams and Cartwright (1963) reported poor reliability and reproducibility of the urinary excretion test of vitamin B12 absorption after partial gastrectomy and in intestinal malabsorptive disease. They found consistently normal or abnormal results in only 2 of 8 partial gastrectomy subjects and in only 1 of 7 subjects with primary malabsorptive disease. Though the results in the present study are not as striking as those of Adams and Cartwright (1963), nonetheless their findings were confirmed in that both normal and impaired absorption results were most often seen in the same subject in these two groups. The differences in both studies were most striking in the partial gastrectomy group.

Adams and Cartwright (1963) carried out many tests (modal number 6) on each subject. Analysis of their results shows that, where normal and abnormal results were obtained, the majority of tests were usually in one category. In only 2 of 12 subjects were

results equally divided between the normal and abnormal ranges. On these grounds, the authors suggested that only a series of absorption tests could indicate whether significant malabsorption was likely to be present.

The reasons for this variability are unknown. After partial gastrectomy, diminished intrinsic factor secretion certainly occurs and is an important cause of vitamin B12 malabsorption (Ardeman and Chanarin 1966). Adams and Cartwright (1963) have in addition suggested that intrinsic factor secretion may be irregular and intermittent after partial gastrectomy. Unduly rapid emptying of the gastric remnant, resulting in poor binding of vitamin B12 and intrinsic factor and little time for the vitamin to be released from food, might also be important. Whatever the explanation, however, this variation in absorption may explain in part the inability of Mahmud et al. (1971) to demonstrate vitamin B12 malabsorption in more than a third of their partial gastrectomy subjects where serum vitamin B12 and even red blood cell vitamin B12 levels were low. A further explanation for the findings of Mahmud et al. (1971) is offered below.

In intestinal malabsorptive disease, variable vitamin B12 absorption may be due to a fluctuating severity of an ileal lesion or to the presence of excess bacteria in the small bowel. In addition, however, concomitant folate deficiency may give rise to vitamin B12 malabsorption (Scott et al. 1968). Five of the 7 subjects studied by Adams and Cartwright (1963) probably had folate deficiency.

The Effect of Food on Vitamin B12 Absorption.

The results of investigations into the effect of food on the absorption of vitamin B12 have been reviewed in the introductory section of this work. In summary, Swenseid et al. (1954) originally showed that absorption of vitamin B12 in normal subjects was improved when it was given with a meal. Deller et al. (1961) could not confirm these results in normal subjects but found that food increased absorption in persons who had had a partial gastrectomy. Turnbull (1967) has more recently confirmed the results of Deller et al. (1961).

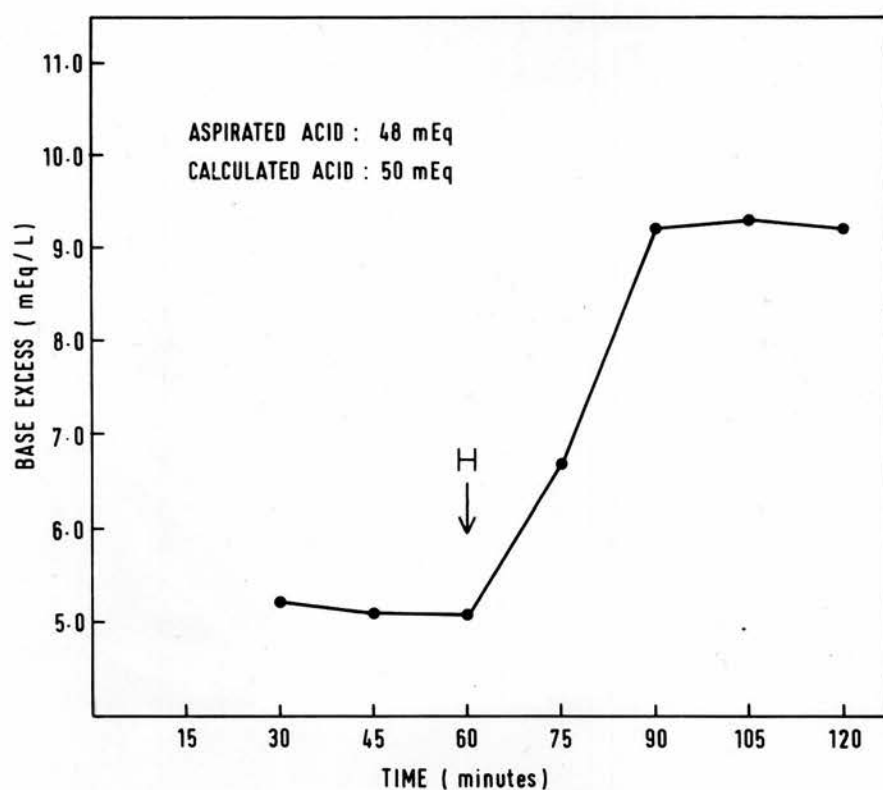
In the work reported above, the mean absorption of vitamin B12 was higher when it was given with a vitamin B12-free meal in control and achlorhydric subjects, but most of all in those who had had a partial gastrectomy. As already pointed out, tests with and without food were not done on the same subjects, and particularly as the groups given tests with food were smaller, it is difficult to know the extent to which the results can be compared. A further note of caution has to be introduced regarding the results in subjects with partial gastrectomy, as absorption tests in this group can give very variable results (Adams and Cartwright 1963), a finding confirmed in this study. However, as all reports so far on subjects with partial gastrectomies have shown increased vitamin B12 absorption in the presence of food, the observation is probably a true one.

This has important implications for studies on the occurrence of vitamin B12 malabsorption after partial gastrectomy, where tests done in the fasting state have indicated a 30-40% incidence (Lous and

Schwartz 1959, Deller et al. 1962, Weir et al. 1966, Hanngren et al. 1967). It has been suggested (Chanarin 1969) that these studies in the fasting state may have overestimated the incidence of vitamin B12 malabsorption under normal conditions and that a reduction of serum vitamin B12 should not be taken as evidence of deficiency. On the other hand, absorption of crystalline vitamin B12 given with food is not the same as absorption of natural vitamin B12 from food, and this makes the above suggestion suspect. Deller et al. (1962) and Mahmud et al. (1971) have found that partial gastrectomy subjects with reduced serum vitamin B12 levels often did not have reduced absorption of the vitamin as assessed by the urinary excretion or the plasma radioactivity test. However, as Mahmud et al. (1971) also showed low red blood cell vitamin B12 levels in 21 of 22 subjects with low serum levels, it is hard to resist the conclusion that these subjects were truly vitamin B12 deficient. It may be that persons who have had partial gastrectomy cannot easily release vitamin B12 from food, but that once released, the presence of food increases its absorption.

The reason for the increased absorption of vitamin B12 in the presence of food is unknown. Swenseid et al. (1954) suggested that food caused the secretion of intrinsic factor, and this may be so, as Rune (1966) has shown that food can stimulate a maximal acid, and presumably therefore intrinsic factor, secretion. This was done by showing that gastric acid secretion could be calculated indirectly from the simultaneous changes occurring in blood base levels, an observation that has been confirmed by the present author, as shown in Fig. 30.

FIG. 30: Change in blood base excess during a gastric function test, showing the correlation between the amount of acid retrieved by nasogastric aspiration and the amount calculated from the base excess changes.



H = point at which histamine 0.04mg/kg of body weight was given parenterally.

Rune found that acid secretion calculated in this way was the same after histamine and after a meat meal. In this regard, Turnbull (1967) showed that histamine was as effective as food in increasing vitamin B12 absorption after partial gastrectomy.

Vitamin B12-free meals, however, are meat-free meals, and whether such food will stimulate much secretion is unknown. An alternative explanation may be that gastric emptying is slower when food is taken, allowing better mixing of the vitamin B12 and intrinsic factor and some neutralisation of the gastric contents, which would result in less peptic destruction of the latter. The findings of Irvine et al. (1970) indicated that pentagastrin did not increase vitamin B12 absorption in control or in achlorhydric subjects, even though it did increase intrinsic factor production in both groups. It may be that after partial gastrectomy the effect of food on increasing intrinsic factor secretion is relatively more important in promoting the vitamin B12 absorption, while in control and achlorhydric subjects its effects on retarding gastric emptying is more important.

Both in this study and in that of Deller et al. (1961), food did not improve vitamin B12 absorption in pernicious anaemia. This would be expected where intrinsic factor secretory capacity was severely restricted.

A comment may be made here on two achlorhydric subjects who showed strikingly different vitamin B12 absorption when tests were done with and without food (Table XXI). The first subject was in good health, with a normal serum vitamin B12 level and good intrinsic

factor secretory capacity. The two absorption tests done in the fasting state were well within the normal range, and those done in the presence of food fell just below the normal range. In this case, absorption was clearly less in the presence of food for no obvious reason. No feasible explanation can be given.

The second subject, also achlorhydric, presented with iron-deficiency anaemia and a marked reduction in intrinsic factor secretory capacity. While still anaemic and before the start of the treatment, two vitamin B12 absorption tests in the fasting state were at or below the lower limit of normal. After correction of the anaemia by iron therapy, absorption tests in the presence of food were well within the normal range. Each pair of tests correlated fairly well. The increase in absorption after treatment was greater than the increase found in any other achlorhydric subject in this study when tests were done with food. It is tempting to postulate, therefore, that the iron deficiency was the cause of a temporary vitamin B12 malabsorption corrected by treatment with iron. Cox et al. (1959) reported that 13 of 25 patients with iron deficiency had low serum vitamin B12 levels which corrected on iron treatment alone, and they suggested that iron deficiency produces vitamin B12 malabsorption, possibly by reducing intrinsic factor secretion. Badenoch et al. (1957) noted an association between malabsorption of vitamin B12 and gastric atrophy in iron-deficient subjects, while Cook and Valberg (1965) showed that iron-deficient subjects with normal gastric acid secretion had normal vitamin B12 absorption. Cook and Valberg (1965) concluded that vitamin B12 deficiency in iron-

deficient states resulted from the presence of independent gastric atrophy and not from iron deficiency per se. This view is supported by the work of Shearman et al. (1966), who found that only the idiopathic form of iron-deficiency anaemia was associated with achlorhydria and the presence of parietal cell autoantibodies; furthermore, iron therapy did not alter the achlorhydric state. In the case discussed here, there was no increase in intrinsic factor secretion after correction of the anaemia (Table XXI). This suggests that some other temporary cause of vitamin B12 malabsorption, corrected by iron therapy, may have been present during the iron-deficient state, possibly in the ileum.

It was found that the reproducibility of sequential tests of vitamin B12 absorption where the test dose was given with food, as opposed to the fasting state, was significantly better for control subjects and for achlorhydric subjects who did not have pernicious anaemia. Where test doses are given in the fasting state, it is likely that the amount of intrinsic factor which happens to be available in the stomach is very variable; in addition, fluid is known to be able to pass through the stomach very quickly. The capacity for variability in the amount of intrinsic factor happening to be present in the gastric lumen would be greatest in these two groups of subjects, accounting in part at least for the poor reproducibility of fasting state absorption tests. The improvement in reproducibility where food was given could be due in part to increased intrinsic factor secretion, resulting in an equalisation of the amount of intrinsic factor available, and in

part to less rapid passage of vitamin B12 from the stomach, allowing greater time for the binding of these two substances.

Summary.

Measurement of vitamin B12 absorption by whole body counting gave identical results in control and achlorhydric subjects. The range of normal absorption is very wide, not only because of variations between subjects, but also because of variations in the same subject. Only 8 of 62 absorption tests on 31 pernicious anaemia subjects fell in the normal range, and only one such subject gave a normal value on two occasions. Most of these normal results were probably due to failure to excrete unabsorbed radioactivity, though variable intestinal malabsorption of the vitamin may have been misdiagnosed by chance. The possibility that normal absorption of vitamin B12 might occasionally occur is discussed. Overall, the test discriminates well between normal or achlorhydric subjects and pernicious anaemia subjects.

In subjects who had had partial gastrectomy or who had intestinal malabsorption, test results in both the normal and abnormal range were often obtained. Diminished and erratic intrinsic factor secretion and variable gastric emptying may be of most importance in producing this in partial gastrectomy. A fluctuating intestinal lesion, possibly with folate deficiency contributing to malabsorption before treatment, may be important in intestinal disease.

The mean absorption of vitamin B12 was greater in controls, achlorhydric subjects, and especially in those with a partial gas-

trectomy when the test dose was given with food. This may be due to increased intrinsic factor secretion due to the food, decreased peptic destruction of intrinsic factor due to food buffering, and slower gastric emptying resulting in better mixing. Food also improves test reproducibility in control and achlorhydric subjects. Comments are made on unusual results in two achlorhydric subjects and on malabsorption after partial gastrectomy.

COMPARISON OF WHOLE BODY COUNTING WITH OTHER METHODS OF MEASURING VITAMIN B12 ABSORPTION

There have been relatively few studies in which whole body counting has been compared with other methods of measuring vitamin B12 absorption. The main studies, in addition to that reported above, are those of Callender et al. (1966), Tappin et al. (1966), and Cottrill et al. (1971).

Callender et al. (1966) showed that there was a very good correlation between the faecal excretion and whole body counting methods, provided that gross discrepancies, accounted for in retrospect by history-taking as being due to inadequate faecal collections, were excluded. Two points were clearly important with respect to these latter. First, they amounted to 16 of 54 collections (29.6%) and, second, their inadequacy would not have been recognised had the whole body counting method not been done as well. These results agree closely with those reported in this study, where 13 of 56 collections (23.2%) were thought to have been inadequate. These two studies, therefore, emphasise that although the faecal excretion method may be a valid

quantitative test of vitamin B12 absorption, subject-related failures are rather common, even under research conditions, and there is no sure way of detecting these failures when only the faecal excretion test is done.

The studies of Tappin et al. (1966) and Cottrall et al. (1971) showed that there was a fairly good correlation between the whole body counting, the urinary excretion, and the plasma radioactivity methods. No evidence on these points was sought in this study, as the giving of a "flushing" dose of nonradioactive vitamin B12 was specifically avoided. However, as judged by the urinary excretion studies reported above, the supposition of these workers is probably true that the percentage urinary excretion in the 24 hours after giving the "flushing" dose, added to the eventual whole body retention, gave a good estimate of the true vitamin B12 retention without a "flushing" dose.

THE RELATION OF INTRINSIC FACTOR SECRETORY CAPACITY TO VITAMIN B12 ABSORPTION

It is now well established that normally intrinsic factor is secreted in very great amounts even under basal (i.e. unstimulated) conditions. According to Ardeman (1965), male control subjects secrete 100-8300ng units/hour (mean 2600ng units) in the basal state, and from 1400 to 21,500ng units/hour on histamine stimulation. Females secrete about half this amount, which is wholly the result of producing a smaller volume of gastric juice. This is far greater than the 400-500ng units needed to restore optimal absorption from 1.0µg of vitamin

Bl2 in subjects with pernicious anaemia (Ardeman and Chanarin 1965). It was for this reason that the relation of intrinsic factor secretion to vitamin Bl2 absorption was studied only in subjects with a limited secretory capacity.

In making this comparison, it is important to recognise that the accuracy of both the intrinsic factor measurement and the estimate of vitamin Bl2 absorption will be at their least. In the intrinsic factor assay used in this work, duplicate samples were measured for a preset count of 4000 counts to determine the relative counting rates. This represents a counting time of about 350 seconds at low intrinsic factor concentrations. Since the intrinsic factor determination depends on the difference between counting rates, the accuracy of the measurement deteriorates at low intrinsic factor concentrations because the count rates come closer together. Even when differences between the duplicate assay samples of no more than 10% were accepted, this meant that the standard error due to statistics could be 50% for very low intrinsic factor concentrations. For illustrative purposes the standard error due to statistics was calculated for three subjects; this and the effect it would have on the calculated total intrinsic factor output after gastric stimulation are shown in Table XXIV. Nonetheless, in computing the total intrinsic factor secreted after gastric stimulation, incomplete gastric juice aspiration was probably as important a source of error as that due to counting. This implies that moderate differences in intrinsic factor output at low levels are probably not significant.

TABLE XXIV: *The effect on the total intrinsic factor output in one hour after gastric stimulation of the standard error due to counting statistics, where the concentration of intrinsic factor was low*

SUBJECT	CALCULATED INTRINSIC FACTOR OUTPUT (ng units/hour)	STANDARD ERROR DUE TO COUNTING STATISTICS (%)	POSSIBLE RANGE OF INTRINSIC FACTOR OUTPUT
1	71	50	36-100
2	140	32	95-195
3	377	9	340-414

For comparable reasons, the whole body counter is also at its least accurate where there is a low vitamin B12 absorption. Warner and Oliver (1966), using a counter similar to that used in this study, found a standard deviation of 10-15% on the final count. Allowing a 5% error on the 100% count, this means a final absorption of 20% could really be anywhere from 16% to 24%. It is important to bear these methodological limitations in mind in this intrinsic factor secretion-vitamin B12 absorption comparison.

In the present study, whether one considered the largest or the smallest amount a subject absorbed in a sequence of tests in the fasting state, X^2 analysis showed a significant relation between the amount of vitamin B12 absorbed and the intrinsic factor secretion in one hour after gastric stimulation. This observation agrees with that of Ardeman and Chanarin (1965), in which the urinary excretion test was used to measure vitamin B12 absorption, and was confirmed by Irvine et al. (1970) using whole body counting tests.

The data of Ardeman and Chanarin (1965), on the other hand, suggested a sharp separation between subjects with pernicious anaemia, who secreted little more than 200ng units of intrinsic factor after gastric stimulation, and those subjects with other conditions. However, few subjects with intrinsic factor secretions of 200-1000ng units were studied, and no achlorhydric subjects without pernicious anaemia. The results of the present study suggest that, despite the significant general relation between intrinsic factor secretion and vitamin B12

absorption, there was considerable overlapping, due both to subjects who secreted little intrinsic factor and yet absorbed normally, and vice versa. Irvine et al. (1970) also found a number of achlorhydric subjects without pernicious anaemia who gave absorption results in the normal range and yet had intrinsic factor secretion levels similar to those with pernicious anaemia. The overlapping of achlorhydric and pernicious anaemia subjects was not obviated by giving the vitamin B12 test dose with food.

These results indicate that, contrary to the suggestion of some authors (Irvine et al. 1968), the diagnosis of pernicious anaemia cannot be made with absolute certainty by measuring intrinsic factor secretory capacity. There is no doubt that all pernicious anaemia subjects have grossly diminished intrinsic factor secretory capacity as compared with controls. Irvine et al. (1968) suggested from their study of 81 subjects with pernicious anaemia that intrinsic factor secretion of greater than 200ng units in the hour after gastric stimulation rarely if ever occurred, irrespective of whether or not antibody to intrinsic factor was present in the serum, and that achlorhydric subjects without pernicious anaemia rarely secreted less than 200ng units in the same circumstances. In a later publication, however, these workers (Irvine et al. 1970) found that 10 of 36 achlorhydric subjects secreted less than 100ng units of intrinsic factor and yet absorbed 22-58% of a 0.5µg dose of vitamin B12; 5 of these 10 subjects secreted hardly any intrinsic factor, and yet all absorbed more than

35% of the test dose by whole body counting. Ardeman and Chanarin (1965) suggested that subjects with pernicious anaemia secreted less than 200ng units of intrinsic factor in the hour after gastric stimulation; but later these workers also found one patient with a normal vitamin B12 absorption test who secreted only 200ng units, and another with a similar secretory capacity whose absorption of vitamin B12 was in the low normal range (Ardeman et al. 1966). The findings in one of these two subjects, however, suggested that intrinsic factor secretion probably varied considerably.

Subjects such as these were found in the present study. In the two subjects reported in Table XXII, intrinsic factor secretion was very low on two occasions during the weeks when vitamin B12 absorption tests were normal. Nonetheless, occasional vitamin B12 malabsorption, or malabsorption of food vitamin, was suggested by low or low-normal serum vitamin B12 levels in both these subjects. These results support those of Whiteside et al. (1964), who found low serum vitamin B12 levels in 4 subjects with gastric atrophy and normal vitamin B12 absorption; these workers also found normal serum vitamin B12 levels in 7 such subjects who showed malabsorption. Irrespective of the interpretation of these findings, however, it is clear that achlorhydric subjects without pernicious anaemia may show intrinsic factor secretory capacities within the pernicious anaemia range and yet absorb crystalline vitamin B12 normally.

It is equally interesting and important that subjects with pernicious anaemia may secrete amounts of intrinsic factor in response

to gastric stimulation well within the range of achlorhydric subjects able to absorb vitamin B12 normally. Three such subjects are reported in Table XXIII, where intrinsic factor secretion ranged from 548 to 825 units/hour after gastric stimulation. All fulfilled the criteria for pernicious anaemia, and none had gross small intestinal disease. Six similar subjects have been reported by Wangel et al. (1968) in whom malabsorption of vitamin B12, reversible with exogenous intrinsic factor, was well documented; these subjects secreted 420-840ng of intrinsic factor after gastric stimulation. There may be many reasons for the occurrence of vitamin B12 malabsorption in subjects such as these, where intrinsic factor secretion, albeit greatly depressed, seems adequate; such factors would include fluctuating intrinsic factor secretion (Ardeman et al. 1966), varying amounts of intrinsic factor antibodies present in the bowel (Rose and Chanarin 1969), or the presence of excess numbers of small intestinal bacteria (Sherwood et al. 1964). Unfortunately, apart from deficient intrinsic factor secretion, no evidence as to other positive causes of the vitamin B12 malabsorption were found in the subjects reported in Table XXIII.

It is important that collecting the small and often viscid amounts of gastric juice generally secreted by achlorhydric subjects may be difficult (Irvine et al. 1970) and failure often results in marked errors due to incomplete collection. Callender et al. (1960) found that the mean volume secreted in pernicious anaemia after histamine was 11.4ml/45 minutes (i.e. 15.2ml/hour) with a range of 0.5-28.8 ml (i.e. 0.8-38.4ml/hour), and Ardeman (1966) 16ml/hour (range 2-52ml).

Neither group found that histamine increased secretion. Rødbro et al. (1965) found a mean secretion of 24ml in pernicious anaemia. The volumes obtained after gastric stimulation in this study are shown in Fig. 27. The mean volume was greater than in the series quoted, being 42ml; this, however, was less than in subjects with achlorhydria, who secreted a mean of 51ml. The large volumes of juice collected after gastric stimulation in pernicious anaemia subjects in this study may account for the occasional subjects with relatively high intrinsic factor outputs.

Summary.

In conclusion, it seems that at low intrinsic factor secretory capacities ($< 1000\text{ng}$ units in one hour after gastric stimulation) there is a significant relation between the intrinsic factor secretory capacity and vitamin B₁₂ absorption. The present study showed that this relation remained even when the results of more than a single test in each subject were considered. However, both in this study and in the work of others, individual subjects may secrete little intrinsic factor and yet absorb vitamin B₁₂ normally, and vice versa. The problems of ensuring complete gastric juice collections where gastric secretory capacity is low may account for some of the former cases. On the other hand, it is likely that once intrinsic factor secretion has diminished considerably, other factors such as the constancy of intrinsic factor secretion and the presence of intrinsic factor anti-

bodies may determine whether or not malabsorption occurs. Such factors may result in malabsorption in pernicious anaemia subjects with relatively high intrinsic factor secretions.

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Studies from the following papers are included in this thesis.

1. Shearman, D.J.C., Finlayson, N.D.C., Murray-Lyon, I.M., Samson, R.R., and Girdwood, R.H. (1967). *Lancet* 2, 192. Intrinsic factor secretion in response to pentagastrin.
2. Finlayson, N.D.C., Shearman, D.J.C., Simpson, J.D., and Girdwood, R.H. (1968). *J. Clin. Pathol.* 21, 595. Determination of vitamin B12 absorption by a simple whole body counter.
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4. Finlayson, N.D.C., Simpson, J.D., and Shearman, D.J.C. (1970). *Scand. J. Gastroenterol.* 5, 261. Whole body counting of vitamin B12 absorption supplemented by profile scanning.
5. Boddy, K., King, P.D., Henderson, J.T., Shearman, D.J.C., Finlayson, N.D.C., Simpson, J.D., and Tothill, P. *Phys. Med. Biol.* (in press). A direct intercomparison of two whole body monitors in the measurement of the absorption of an oral dose of vitamin B12 labelled with ^{57}Co and ^{58}Co .